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UNITED STATES DEPARTMENT OF THE INTERIOR
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UNITED STATES DEPARTMENT OF THE INTERIOR
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THE

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CHANGES IN WARM-WATER PONDS AFTER HYDRATED-LIME APPLICATIONS

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THE IMPORTANCE OF CALCIUM COMPOUNDS IN INFLUENCING BIOLOGICAL PRODUCTIVITY OF FRESH WATERS has been pointed out by limnologists and fishery workers on numerous occasions (Schaeperclaus 1933; Ohle 1934; Juday, Birge, and Melocke, 1938; Neess 1949; Mortimer and Hickling 1954).

A study of the European literature on pond fertilization indicates that the use of periodic applications of some form of calcium has become fairly standard practice in European countries where pondfish culture is carried on (Neess 1949, Mortimer and Hickling 1954). The value of adding calcium or lime has been described by Schaeperclaus (1933) as being beneficial from a twofold standpoint. The health of fish in a pond is protected when a caustic form of lime is applied, because the treatment kills parasitic or competitive organisms, creates a slightly alkaline reaction to the water (pH 7-8) which is favorable to the well-being of fish, and improves conditions for operation of the metabolic cycle. Also, the productivity of limed waters is increased because extreme pH changes are prevented, a carbonic acid reserve is established, bacterial activity in converting organic material is accelerated, base exchange of nutrients in the bottom mud is facilitated, the danger of oxygen shortages is minimized through holding down excessive putrescible substances, and adequate calcium for favorable development of aquatic plants and animals is provided.

In the United States, only limited use has been made of European research on the employment of lime. Swingle and Smith (1938) recommended that basic slag be added to the fertilizer mixture applied to farm ponds where the nitrogen source was the highly acid ammonium sulfate. Later work by Swingle (1947) revealed that the application of ground limestone to ponds fertilized with organic materials, urea, ammonium sulfate, sodium nitrate, and a commercially mixed inorganic fertilizer resulted in reduced production of fish in every pond where it was used.

Hydrated lime was used by Hasler, Brynildson, and Helm (1951) to alkalize acid bog lakes in Wisconsin. Conditions for trout growth were improved as stained water cleared and allowed more plant growth to occur, although Johnson and Hasler (1954) later reported that there was no apparent increase in fish production in the limed waters.

Zeller and Montgomery (1958) reported that the addition of basic slag or ground limestone to 22 soft-water farm ponds in Georgia resulted in improving the plankton growth, raising the pH, and increasing the total-hardness readings. Prior to liming, all were considered to be problem ponds which did not produce satisfactory phytoplankton growth after the application of inorganic fertilizers.

In spite of the extensive European research on the use of lime as a sterilant and as a fertilizer, there are few published data in regard to the immediate

effects of lime added to the pond environment. Ebeling (1931) applied calcium oxide (quicklime) to ponds at a rate of about 1,400 pounds per acre, spreading the application over a 9-day period. The measured pH of water samples from the treated ponds increased from 7.5 to 9.0, and this was accompanied by a noticeable decrease in total alkalinity. Ohle (1938) pointed out that the pH levels may rise above 10 in recently limed waters. These limited reports offer little help to the fish-culturist in predicting the results of applying specific amounts of lime to a pond.

TABLE 1.--Analysis of water supplies

Well number	4	5	9
Ponds supplied[1/]	1, 2, 3, 4, 5, 9, 12	11, 13, 6, 7	13, 14
Temperature (°C.)	19.1	19.0	21.2
pH	7.5	7.0	7.9
Free CO_2 (p.p.m.)	8.0	21.0	3.0
$^{-}CO_3$ (p.p.m.)	0.0	0.0	0.0
$^{-}HCO_3$ (p.p.m.)	120.0	99.0	79.0
Calcium (p.p.m. as $CaCO_3$)	62.0	47.0	74.0
Magnesium (p.p.m. as $CaCO_3$)	36.0	32.0	34.0
Total hardness (p.p.m. as $CaCO_3$)	98.0	79.0	108.0
PO_4 (p.p.m.)	0.015	0.055	0.015

[1/] Pond 14 was supplied from either Pond 13 or Well 9, depending upon the supply available.

An earlier paper by Snow and Jones (1959) described experiments in the use of ground limestone ($CaCO_3$) and quicklime (CaO) in warm-water hatchery ponds. Indications were that conditions for bluegill production were improved when either of these forms of calcium was applied. Tested in the laboratory, hydrated lime ($Ca(OH)_2$) compared favorably with quicklime as to alkalizing effects and appeared to be safer for general use because it was less irritating to the skin of personnel who handled it.

An experiment was conducted to test the feasibility of adding enough hydrated lime to ponds filled with water to produce a theoretically neutral pH in the bottom mud and to observe the effect of added lime on the pond bottom, the water contained in the pond, the bottom fauna, and the aquatic vegetation present.

Twelve ponds were chosen in the same general area of the Marion, Alabama, National Fish Hatchery. The sizes of the ponds ranged from 0.8 acre to 2.9 acres. Water was supplied to the ponds from three artesian wells. Analyses of water samples from these wells are listed in table 1.

The ponds were divided into three groups of four ponds each. Assignment to a group was based on the pH of bottom-soil samples that were collected while the ponds were drained.

Most of the quoted research indicated that combined lime and phosphate addition gave the best results in carp culture. As much of the European work recommended that lime and phosphate application be combined, an attempt was made to secure information on the results of liming ponds to a pH of 7.0 with hydrated lime followed by the addition of superphosphate at a rate of 8 pounds of phosphorus pentoxide (P_2O_5) per acre every 2 weeks from mid-July to early in November, a total of nine applications.

The experimental ponds were filled with water during the spring. Hydrated lime was spread by hand over the surface of the pond on July 1 and 2. The amount applied was based on the lime requirements for each pond as indicated by the analysis of bottom-soil samples which had been taken the previous winter while the ponds were drained. Six representative sampling stations per acre were used. At each station a bottom-soil sampler constructed from plans described by Walker (1955) was used to take a sample of the pond bottom soil down to a depth of 2 inches. The samples taken from a pond

TABLE 2.--Treatment data and long-range effects on bottom soil of adding lime

Pond No.	pH 12/18/56	Lime requirements as $CaCO_3$	Treatment	pH 1/23/58	pH 1/19/59
1	7.8	None	None (Control)----------	7.6	7.5
2	4.7	3,400	None (Control)----------	5.5	5.2
5	6.3	1,400	None (Control)----------	7.2	7.0
6	6.1	1,350	None (Control)----------	7.2	5.8
4	6.1	600	444 lbs. $Ca(OH)_2$ per acre----------	7.5	7.4
7	5.6	1,900	1,406 lbs. $Ca(OH)_2$ per acre----------	7.2	5.4
9	5.1	3,500	2,590 lbs. $Ca(OH)_2$ per acre----------	7.2	4.7
12	5.1	2,600	1,924 lbs. $Ca(OH)_2$ per acre----------	7.1	6.2
3	6.9	500	370 lbs. $Ca(OH)_2$ per acre plus P_2O_5----	7.4	7.4
11	6.7	900	666 lbs. $Ca(OH)_2$ per acre plus P_2O_5----	7.3	6.2
13	4.8	3,550	2,627 lbs. $Ca(OH)_2$ per acre plus P_2O_5--	7.4	6.3
14	5.1	2,450	1,813 lbs. $Ca(OH)_2$ per acre plus P_2O_5--	7.3	6.4

were combined to make a composite pond sample. The lime requirement for this sample was determined by Auburn University's Soils Testing Laboratory, which used a modification of the buffer method described by Woodruff (1947).

As the Soils Testing Laboratory normally gave lime requirements in terms of ground limestone or calcium carbonate, its lime-requirement poundages were reduced by 26 percent to compensate for the relatively higher amount of calcium in hydrated lime than in calcium carbonate. Table 2 lists the amounts of hydrated lime applied as well as the ponds that received the phosphate treatment.

Ponds were stocked July 29-31 with 165 bluegills (average weight, 0.05) per acre. The ponds were drained and the fish crop was harvested during the period November 6, 1957, to January 13, 1958. Effects of the lime applications on fish production could not be determined because several ponds were contaminated with other fish species which entered the pond system in some way.

Effects of Lime on Pond Water and Bottom Mud

Bottom-soil samples were taken the winter prior to treatment and again about 6 and 18 months after treatment according to the procedure already described. In addition, samples were collected 4 days before liming and again 25, 35, and 100 days after lime was applied with the ponds full of water. The latter samples were taken as described earlier, except that they were from a depth of only 0.5 inch instead of 2 inches. A Beckman pH meter with a glass electrode was used to measure the pH of these samples. Table 3 contains results of the pH determinations.

The pH data of the soil samples taken just before and several weeks after treatment indicated that prolonged drastic pH changes did not occur. Unfortunately it was not possible to secure samples of the bottom soil for determination of the pH which existed immediately after liming, but those taken 25 days after liming did not indicate that measurable changes occurred.

The objective of the lime treatment was to obtain a pH of 7.0 in the bottom soil. After 25 days, two (Nos. 3 and 4) of the eight ponds that received lime had a pH of 7.0 or higher, two ponds were virtually unchanged, and four were more acid than before lime was added.

The samples taken 35 days after liming showed a trend toward greater alkalinity, although the change was not marked. Two

of the ponds that were limed were more acid than before treatment, five were more alkaline, and one was unchanged. Two of the unlimed ponds were more acid, one was less acid, and one was unchanged.

After 100 days, the soil samples taken were generally slightly more acid than they had been on the previous sampling date. Two of the unlimed ponds were more acid; two were more alkaline. Seven of the eight limed ponds were more acid, though the increase in acidity was not great. Further changes apparently occurred during the fall and winter, either before or after the ponds were drained. Samples were collected from the drained pond bottoms about 6.5 months after treatment with lime. In the limed ponds, all samples were close to pH 7.0, which had been set as a desirable level to be established. The range of variation between ponds was from pH 7.0 to pH 7.4. This slow response to lime was in general agreement with the findings of Thompson (1957), who stated that about 1 year was required for added limestone to make a significant change in soil pH when applied to cropland. Three of the unlimed ponds also were more alkaline than they had been before treatment, and samples from Pond 2 showed a change of 0.7 pH.

TABLE 3.--Changes in bottom soil pH before and after liming

Pond number	Pounds $Ca(OH)_2$ added	pH of 1/2-inch-deep soil sample			
		4 days before	25 days after	35 days after	100 days after
1	None	6.7	7.4	7.2	6.9
2	None	5.9	6.2	5.5	6.3
5	None	6.9	6.3	6.2	6.3
6	None	6.7	6.9	6.7	6.2
4	444	6.9	7.3	7.1	6.9
7	1,406	6.5	5.8	6.3	6.8
9	2,590	5.3	5.4	6.3	6.0
12	1,924	6.0	5.8	5.9	5.8
3	370	7.0	7.3	7.2	7.1
11	666	6.4	6.4	6.4	6.3
13	2,627	6.1	5.9	6.2	6.1
14	1,813	6.2	5.8	6.6	6.3

Soil samples were collected 1 year after those discussed above, i.e., about 18 months after liming. Analysis by the same laboratory for the pH showed that samples from two of the unlimed ponds were more acid than samples made 18 months earlier had been, though two were slightly more alkaline. Samples from the limed ponds were considerably more acid than they had been the winter after treatment, and the ponds--with the exception of the two that received the lowest amounts of lime--appeared to be reverting to the original acid condition. Samples from three ponds showed a more acid pH than the samples which had been collected from those ponds before the addition of lime.

Chemical Changes in Pond Waters

Water samples were collected from all ponds in the experiment 3 days before and at intervals after liming. Oxygen content was determined by the Winkler method. Carbon dioxide, bicarbonates, carbonates, and hydroxides were determined by the procedure described by Swingle and Johnson (1953), except that laboratory measuring procedures were used for samples and reagents. The pH measurements were made with a Beckman pH meter. Total hardness and calcium was determined by means of the Diehl, Goetz, and Hach procedure. Total alkalinity and magnesium were obtained by calculation.

Table 4 lists the changes in pH, $^{=}CO_3$, $^{-}HCO_3$, and ^{-}OH which resulted from the application of varying amounts of hydrated lime to these ponds to produce a soil pH of 7.0. Prior to treatment, all ponds contained moderate to heavy growths of rooted vegetation. Consequently, even though all water samples were taken before sunrise to minimize the effect of plant photosynthesis, pH readings were above pH 9, and an appreciable concentration of carbonate was present.

TABLE 4.--Changes in pH, carbonates, bicarbonates, and hydroxides in water samples from several rates of lime application

Pond No.	Amount lime[1] per acre	pH		$^{=}CO_3$		$^{-}HCO_3$		^{-}OH[3]		Depth of sample
		Before[2]	After	Before	After	Before	After	Before	After	
3	370	9.6	10.2	17.1	26.7	51.2	0.0	0.0	6.5	Surface
		9.5	10.1	15.0	28.5	51.9	14.3	0.0	0.0	Bottom
4	444	9.2	10.1	12.9	26.4	40.9	0.0	0.0	1.5	Surface
		9.0	10.0	8.7	24.6	55.5	6.7	0.0	0.0	Bottom
[4]11	666	9.4	10.3	10.5	19.8	35.4	0.0	0.0	15.7	Surface
7	1,406	9.6	10.7	16.5	22.5	32.3	0.0	0.0	41.0	Surface
		9.2	10.5	7.5	47.1	55.8	0.0	0.0	18.0	Bottom
14	1,813	9.3	10.9	8.1	25.5	37.5	0.0	0.0	65.0	Surface
		9.2	11.7	5.4	39.3	41.2	0.0	0.0	456.0	Bottom
12	1,924	9.1	10.9	5.4	24.3	35.4	0.0	0.0	74.5	Surface
		9.1	11.7	4.8	51.0	36.9	0.0	0.0	507.0	Bottom
9	2,590	9.6	11.2	14.7	32.4	24.4	0.0	0.0	123.0	Surface
		9.4	11.9	13.8	43.6	27.1	0.0	0.0	951.0	Bottom
13	2,627	9.3	11.2	7.8	28.5	36.6	0.0	0.0	153.0	Surface
		9.3	12.0	8.1	46.5	36.9	0.0	0.0	1,124.0	Bottom

[1] As hydrated lime, $Ca(OH)_2$.

[2] Samples were taken 3 days before and 1 day after lime was applied.

[3] As $CaCO_3$ equivalent.

[4] Data on bottom sample discarded because of a discrepancy between pH and $^{-}HCO_3$ values which indicated that one of the readings was in error.

After the application of hydrated lime, the pH became more alkaline in all limed ponds, although not in direct proportion to the quantity of lime applied. Carbonates increased, bicarbonates decreased, and the hydroxyl ion was present in substantial quantities in the ponds that received the higher rates of lime. Hydroxides were present only in the surface waters of the lightly treated ponds, but were higher in the bottom samples than in the surface samples from the ponds receiving more than 1,500 pounds of hydrated lime per acre.

In the ponds most heavily treated, alkalinity was great enough to kill both plant and animal life. As this organic material decomposed, carbon dioxide was liberated. This neutralized hydroxyl ions and precipitated carbonates, thus bringing about a gradual removal of the hydroxides. Dissolved oxygen concentrations dropped to zero in the ponds most heavily treated. A period of 7 to 14 days was required for the neutralization of hydroxide ions and the restoration of normal chemical conditions.

Where moderate to low amounts of hydrated lime were applied, chemical changes were noticeable but not drastic and of short duration. It appeared that fish and other animal life could have survived, in part at least, in Ponds 3, 4, and 11. Four days after liming, hydroxyl ions

were absent and pH was near normal in the pond that received 1,406 pounds of calcium hydroxide per acre.

Effect of Lime Treatments on Aquatic Vegetation

The effects of hydrated-lime applications on aquatic vegetation were observed at weekly intervals before and after the lime was applied. Submerged rooted vegetation was the dominant type of plant growth at the time of treatment. Small quantities of lime such as were applied to to Ponds 3, 4, and 7 had no drastic effects on the rooted aquatics, even though an adverse effect was noted 3 days after treatment. Where more than 1,800 pounds of hydrated lime per acre was applied, however, burning appeared to be severe; and there was a significant reduction in the amount of plant growth as the amount of lime applied was increased. In Pond 13, where 2,627 pounds of lime per acre was applied, a complete kill of all rooted aquatic vegetation resulted. Rapid decay of the plants was followed by the development of phytoplankton.

TABLE 5.--Light penetration measurements

Pond No.	Treatment	Average[1/] light penetration (inches)	Range (inches)
1	No lime----------	36+	36+
2	No lime----------	31.5	19 - 36+
5	No lime----------	33.8	25 - 36+
6	No lime----------	31.6	22 - 36+
Average of all readings		33.2	--------
4	Lime for pH 7.0--	36+	36+
7	Lime for pH 7.0--	28.4	16 - 36+
9	Lime for pH 7.0--	19.1	11 - 36+
12	Lime for pH 7.0--	27.6	21 - 36+
Average of all readings		27.8	--------
3	Lime plus P_2O_5---	29.7	12 - 36+
11	Lime plus P_2O_5---	27.4	8 - 36+
13	Lime plus P_2O_5---	25.6	10 - 36+
14	Lime plus P_2O_5---	19.9	10 - 36
Average of all readings		25.7	--------

[1/] Includes 14 readings taken during the period July 16 to November 5.

Beginning 2 weeks after lime applications, readings of light penetration were taken in the 12 ponds of this experiment in order to estimate the comparative abundance of phytoplankton in the various ponds. A Secchi disc attached to a yardstick was used for this measurement. Values of more than 36 inches were recorded as "36 inches plus" and averaged as 36 inches.

Table 5 shows the average depth of light penetration in the ponds, along with the range of readings taken over a 4-month period. Because of seepage into drain lines, considerably more water was required in Ponds 1, 3, and 4 to maintain a constant level than in other ponds in the experiment. Pond 11 was also subjected to an excessive water exchange, as the water for Ponds 13 and 14 passed through it after leaving the supply well. The small number of replications in each treatment precluded a measurement of the effects of an excessive rate of water exchange in these ponds.

All ponds were treated with sodium arsenite at a rate of 4 p.p.m. As_2O_3 for the control of rooted aquatic vegetation. Arsenic was applied in mid-July and again early in September. The ponds received the arsenic whether the vegetation required treatment or not. A uniform method and rate of application were used.

It appeared that the addition of lime resulted in increased growth of phytoplankton in the treated ponds. Further increase resulted from the addition of phosphorus pentoxide after liming, though the latter increase was not so marked as the difference between limed and unlimed ponds.

Effects of Lime Treatments on Bottom Fauna

Five of the eight limed ponds were selected for a study of the effect of the application of several rates of hydrated lime on the bottom fauna. The ponds included were Nos. 3, 4, 7, 13, and 14. Table 2 shows the amounts of hydrated lime applied. The rates ranged from 370 pounds per acre in Pond 3 to 2,620 pounds per acre in Pond 13.

Prior to sampling the pond bottoms, random locations in deep, intermediate, and shallow water were marked. As the depths of the ponds ranged from 1.5 to 5 feet, the differences in depth were not great. Bottom samples were taken from the marked locations with an Eckman dredge. Three dredge hauls from each location were combined to make a sample. The bottom soils of the ponds ranged from sandy clay to clay overlaid with silt. The presence of rooted aquatic vegetation tended to interfere with the collection of a uniform sample of bottom mud; but as all ponds could be sampled by wading, jaws of the dredge were seated by hand before tripping. Any partial loads were discarded, and a fresh haul was made.

Each location sample was washed through a No. 14 (1,410-micron opening) and a No. 30 (590-micron opening) standard sieve screen. Samples were examined while fresh, and most of the work was completed within 48 hours of collection. Seven groups of organisms were abundant enough and had a wide enough distribution to be used to indicate the effects of different amounts of lime. These organisms were aquatic earthworms, Oligochaeta; damselfly nymphs, Zygoptera; dragonfly nymphs, Anisoptera; midge larvae, Tendipedidae; mayfly nymphs, Ephemeroptera; phantom midges, Chaoborus; and two genera of snails, Gyraulus and Physa.

During the week prior to treatment and after the ponds had been filled several months, samples were taken from the five ponds studied. Four to eight days after lime was applied, samples were taken again from the same locations. Three weeks after liming, a sample was taken from the intermediate-depth location in the five ponds in order to provide comparative data on the return of organisms. The ponds were sampled again late in September and early in November.

The number of organisms noted in each sample collected before and shortly after treatment is given in table 6. Data on the rate of recovery of the index organisms are listed in table 7.

The hydrated-lime applications eliminated practically all of the organisms used as indicators except those in the two ponds that received the lightest amounts. Here there was some survival of forms which appeared to be resistant to the artificially produced alkalinity. The forms enumerated made a rapid recovery, especially noticeable in the heavily limed ponds. In some instances there were more organisms present in the sample collected after 3 weeks than in the samples taken before lime treatment, even though the ponds had been filled far enough in advance for animal populations to become well established. This was particularly true of midge larvae.

The post-treatment samples examined indicated that there were no snails in the samples from four of the five limed ponds (including Pond 3, which received 370 pounds of hydrated lime per acre). Although there was no appreciable reduction in Pond 4 (where 444 pounds per acre had been applied) 1 week after treatment, these results suggest that hydrated lime applied at a rate of about 500 pounds per acre might be useful as a snail-control measure in water having characteristics similar to those of ponds in this experiment. Such treatment apparently would be of temporary value, however, as samples taken from Ponds 7 and 11 contained a few snails late in September. By November, all of the treated ponds that were sampled except No. 14 appeared to have a normal number of snails.

Discussion and Conclusions

The effects of hydrated-lime ($Ca(OH)_2$) additions made to produce a theoretical

TABLE 6.--Effect of lime on the numerical abundance of bottom fauna

Organisms	Time of collection	Pond number 3 D	3 I	3 S	4 D	4 I	4 S	7 D	7 I	7 S	13 D	13 I	13 S	14 D	14 I	14 S
		(Number per sample)														
Aquatic earthworm-----	Pre-treatment---------	0	0	0	50	58	84	12	7	1	15	0	0	0	0	0
	Week after treatment--	0	0	0	43	38	12	0	0	1	0	0	1	0	0	0
	3 weeks after---------	---	1	---	--	39	--	---	6	--	---	1	--	---	0	--
Damselfly nymph-------	Pre-treatment---------	2	0	1	0	1	0	0	2	0	0	0	2	1	0	0
	Week after treatment--	0	1	1	3	0	1	0	0	1	0	0	0	0	0	0
	3 weeks after---------	---	6	---	--	6	--	---	0	--	---	0	--	---	0	--
Dragonfly nymph-------	Pre-treatment---------	1	1	3	0	0	1	4	6	0	0	0	1	5	2	1
	Week after treatment--	2	0	0	2	0	0	0	0	0	0	0	0	0	0	0
	3 weeks after---------	---	0	---	--	1	--	---	0	--	---	1	--	---	2	--
Mayfly nymph----------	Pre-treatment---------	5	13	34	0	4	12	0	3	0	88	67	17	31	45	23
	Week after treatment--	0	0	1	37	0	5	0	0	0	0	0	0	0	0	0
	3 weeks after---------	---	10	---	--	9	--	---	1	--	---	1	--	---	3	--
Midge larvae----------	Pre-treatment---------	12	0	9	0	38	9	2	2	4	6	17	0	0	0	0
	Week after treatment--	0	1	0	4	0	2	0	0	0	0	0	0	0	0	0
	3 weeks after---------	---	8	---	--	24	--	---	7	--	---	24	--	---	2	--
Phantom midge larvae--	Pre-treatment---------	0	3	0	1	0	1	4	7	1	1	2	0	2	1	0
	Week after treatment--	2	2	3	2	0	0	0	0	0	0	0	0	0	0	0
	3 weeks after---------	---	5	---	--	8	--	---	32	--	---	85	--	---	9	--
Snails----------	Pre-treatment---------	593	588	346	65	2	7	207	226	10	454	289	3	164	13	7
	Week after treatment--	0	0	0	74	3	0	0	0	0	0	0	0	0	0	0
	3 weeks after---------	---	0	---	--	0	--	---	0	--	---	0	--	---	0	--

D = Deep sampling location. I = Intermediate depth location. S = Shallow depth location.

TABLE 7.--Comparative number of organisms from limed and unlimed ponds several months after treatment

Pond number	Number of organisms							
	Aquatic earthworm		Damselfly nymph		Dragonfly nymph		Midge larvae	
	9/24	11/8	9/24	11/8	9/24	11/8	9/24	11/8
1	24	3	12	0	10	4	83	99
2	0	30	0	1	2	0	41	517
3	0	1	0	2	1	4	169	430
4	0	1	0	0	0	3	240	16
7	1	13	1	0	9	0	73	206
11	11	46	3	7	5	1	249	159
13	17	3	1	0	0	0	115	251
14	5	69	0	0	2	5	185	1,009

Pond number	Number of organisms					
	Mayfly midge		Phantom midge		Snail	
	9/24	11/8	9/24	11/8	9/24	11/8
1	1	0	26	16	17	1
2	0	0	13	5	29	28
3	0	0	99	80	0	2
4	0	17	27	0	0	1
7	4	0	1	26	2	4
11	36	4	64	203	1	162
13	0	0	141	9	0	43
14	0	0	52	39	0	0

bottom-soil pH of 7.0 were studied over a 3-year period. Use of specified amounts of lime did change the bottom-soil pH to 7 or slightly higher within a 6-month period in all the ponds treated. Changes in the bottom soils were not rapid, even though drastic chemical reactions occurred in the water of ponds where quantities in excess of 1,800 pounds of hydrated lime per acre were added. A high degree of hydroxide alkalinity was noted shortly after the lime was applied, with pH readings as alkaline as 12.0 being measured 1 day after liming.

The effect of lime on the bottom soil appeared to be temporary, as the highly acid ponds were returning to their former state of acidity 18 months after being limed to a neutral condition.

Use of lime in alternate years is indicated in ponds like Ponds 9, 12, 13, and 14 (where a highly acid condition existed before liming) if a pH of 7.0 is to be maintained. In ponds where little lime was needed initially (as in Ponds 3 and 4), the bottom-soil pH remained more stable after treatment with lime.

Higher rates of liming eliminated practically all bottom organisms and higher vegetation in the ponds. Bottom samples taken from a pond a week after application of 1,810 pounds of hydrated lime per acre contained no living organisms big enough to be seen with the unaided eye. Rooted aquatics also were killed in this pond. Aquatic life recovered rapidly, however, as a bloom of phytoplankton was seen in a few days, and some bottom organisms--dragonfly nymphs, mayfly nymphs, and midge larvae--were found in samples collected 3 weeks after treatment.

Phytoplankton, as measured by increased pond-water turbidity, was more

abundant in the limed ponds than in the unlimed ones and was increased further by the addition of superphosphate.

Woodruff's (1947) method of determining lime requirements for soils seems effective in determining the lime requirement of pond-bottom soils if the pH change measured in samples taken 6 months after liming can be used as a criterion.

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INFECTIOUS PANCREATIC NECROSIS OF TROUT
II. EXPERIMENTAL INFECTIONS WITH BROOK TROUT

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THE FIRST REPORT IN THIS SERIES described the results of a tissue-culture study of infectious pancreatic necrosis (Wolf, Dunbar, and Snieszko, 1959). The research showed that fish afflicted with this disease always harbored a submicroscopic agent which could be propagated only in specific kinds of fish tissue culture. The agent produced a characteristic degeneration in tissue culture, was unaffected by antibiotics and high concentrations of glycerin, and for these and other reasons was concluded to be a virus.

Thus far, 10 outbreaks of the disease have been investigated by us, and in every instance the presence of a filterable agent has been demonstrated. Although highly significant, such data did not establish the cause of the disease. Victims of infection often harbor more than one kind of microbe, and the secondary invader may be the more easily recognized. Also, presumably pathogenic microbes may be present without producing a recognizable disease. A series of logical steps, formally termed Koch's postulates, has been established to test the relation of a microbe to a host. Indisputable proof that a particular microbe causes a particular disease is obtained only after demonstrating all of the following: (1) the presence of the microorganism in every case of the disease, (2) isolation of the agent and propagation in pure culture, (3) infection of a suitably susceptible host by a pure culture of the organism, and (4) recovery of the specific microorganism from experimentally infected hosts.

The first two of these postulates were fulfilled in the tissue-culture study. The purpose of this work, therefore, was to complete, if possible, the remaining steps of Koch's postulates and thereby establish the cause of pancreatic necrosis.

Experimental Infections With "Laboratory" and "Wild" Virus

Brook trout (Salvelinus fontinalis) fry of the State fish hatchery at Beaver Creek, Maryland, were used as the susceptible hosts in two separate infection trials.

First Experiment

Methods.--Fry were kept at 12.5° C., and were fed a bound diet of beef liver and spleen combined with about 2 percent salt and 10 percent of a mixture of dry meals (consisting of 2 parts fish meal, 1 part cottonseed meal, and 1 part wheat middlings). When they were 4 weeks old, the fry were distributed at the rate of 100 per 4.25-liter jar. Two jars of fish served as controls and were not exposed to infection. Two other jars were fed four times on the 1st day, and again on the 12th day, with food which contained material from the 8th tissue-culture transfer of the virus.[1/]

[1/] Relative infectivity of new isolants of the virus was routinely assayed by inoculating cultures of explanted trout fin

One month after the first inoculation, the survivors were pooled and redistributed. All fry were then fed three times with homogenized victims of pancreatic necrosis and held for an additional 25 days. The virus used was "wild" virus, as opposed to the laboratory-grown virus, and--to the best of our knowledge--was the most virulent material available. All fish were observed for abnormal behavior characteristic of the disease, and samples of the dead fish were examined histologically for necrosis of the pancreas.

Results.--During the first 5 days of the experiment, unexplained mortality claimed about 4 percent of the fish. These losses probably represented the usual attrition seen in small fish and deaths that were caused by or precipitated by handling and distribution. Coincident with the 6- to 8-day incubation period for pancreatic necrosis, mortality in the infected lots rose. The typical whirling was evident in the jars of infected fish, and examination of the internal organs revealed an absence of food but the presence of visceral petechiae and mucuslike material in the stomach. Necrosis of the pancreas was positively identified in the histological examination. Explant-type tissue cultures were prepared and inoculated with a bacteria-free filtrate of homogenized victim fish. Tissue cultures that were inoculated with this material degenerated very rapidly and in the manner characteristic of infection by the virus of pancreatic necrosis. As far as could be determined, pancreatic necrosis did not occur among the control fish. At termination, 23 percent of the inoculated fish had died and 1 percent of them were unaccounted for. A total of 8.5 percent of the control fish had died (table 1).

Pooled survivors proved to be equally susceptible to infection from the feeding of homogenized victims of pancreatic necrosis, and more than half died during the 25-day observation period that followed the feeding of virulent material (table 1).

Second Experiment

Methods.--Confirmation of results from the first trial was sought in a second and larger experiment. The fry were 8 weeks old and were kept at the same temperature and fed the same diet as before. In order to minimize handling, the fish were used on a weight basis and were not counted at the start of the experiment. Three troughs, each containing 53 grams of fish (about 190 fish), were used for each of the four divisions of the experiment: (1) uninfected controls, (2) infection of fish by two feedings from a pool of homogenized victims of pancreatic necrosis, (3) infection of fish by two feedings of material from the same pool but after it had been filtered to remove bacteria, and (4) infection of fish with two feedings of tissue-culture material of sixth transfer containing about 1/100-millionth of what had first been used to inoculate the first tissue culture. All the lots were observed for 35 days, after which survivors of each division were counted and then pooled. Forty survivors of each division excepting the fry which had been fed unfiltered pool of virus were selected and redistributed at the rate of 20 per 4.25-liter jar. All were given three feedings of homogenized victims of pancreatic

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tissue with serial 10-fold dilutions of homogenized victim fish. Infectivity of such material was lacking in part or in all cultures inoculated with a 1 x 10^{-6} dilution--a dilution which contains only 1/1-millionth of the starting concentration. In preparation for the transmission trials, two strains of the virus were transferred repeatedly. This was done to make certain that infection, if achieved, would be initiated by newly produced virus and not by that virus which had been carried over from the first inoculations. Eighth tissue-culture transfer material had been taken from cultures which had shown viral degeneration. This material was equivalent to a dilution of more than 2.5 x 10^{-10}; that is, it contained less than 1/25-billionth of the original homogenate. As this dilution greatly exceeded the extinction point of the original material, the degeneration and hence the fish infections were probably caused by newly produced virus.

TABLE 1.--Mortality in the first experimental transmission of infectious pancreatic necrosis

Initial exposure to tissue culture-propagated virus (4-week-old fry)				Follow-up infection of survivors by feeding homogenized victim fish (9-week-old fry)			
	Number of fry per vessel	Cumulative mortality			Number of fry per vessel	Cumulative mortality	
		Number	Percentage			Number	Percentage
Controls--------	100	9	9	Previously uninfected controls	90	45	50
	100	8	8		90	58	64.4
Mean-------	------	8.5	8.5				
				Mean--------	------	51.5	57.2
Infected with tissue-culture material------	100	18	18	Previously infected with tissue-culture material-------	75	40	53.3
	100	28	28		75	45	60.0
Mean-------	------	23	23				
				Mean--------	------	42.5	56.7

necrosis and observed an additional 25 days. Again, all fish were observed for abnormal behavior, and samples of those which died were examined histologically.

Results.--Owing in part, perhaps, to the fact that the fish had not been counted and handling had been minimal, the unexplained mortality (that which occurred in the controls) was less than one-fifth of what it had been in the first experiment. For the same reason, however, data on the unaccounted losses could not be obtained. The results of the second trial confirmed those obtained with the younger fish (table 2). Additional evidence for the filter-passing ability (the so-called filterability) was also obtained. One week after inoculation by feeding, the typical whirling was observed in all troughs of infected fish, and it was followed by a rapid rise in mortality. Peak mortality occurred 2 weeks after infection, and the disease was considered to have run its course at the end of 35 days. At that time more than five-sixths of the fish which had been fed the unfiltered homogenate had died. The filtered homogenate produced less mortality; least mortality occurred in the trio of troughs which had been fed the tissue-culture-propagated virus. A little less than one-third of this group died. Control fish had less than 2 percent mortality. Histological examination of samples of the dead fish showed that pancreatic necrosis was present in all of the infected lots, but it was not found in the controls.

The fry were 13 weeks old when they were given the follow-up inoculation. It resulted in the death of only 2 out of 40 fish in each of the 2 lots with the history of previous infection. Among the 13-week-old fish which had not been infected previously, the same inoculum resulted in the death of 6 of the 40 fish during the 25-day observation period.

Discussion

It has been found that as trout fry grow older, they become increasingly resistant to this disease (Snieszko et al. 1959). This phenomenon was evident in the results of this investigation. Crude

TABLE 2.--Mortality in the second experimental transmission of infectious pancreatic necrosis

Initial exposure to virus (8-week-old fry)				Follow-up infection of survivors by feeding homogenized victim fish (13-week-old fry)			
	Number of fry per trough	Cumulative mortality			Number of fry per trough	Cumulative mortality	
		Number	Percentage			Number	Percentage
Controls--------	164	3	1.8	Previously uninfected controls	20	3	15.0
	195	5	2.6		20	3	15.0
	186	1	.5				
Mean-------	------	3	1.6	Mean--------	------	3	15.0
Infected with tissue-culture material------	185	65	35.1	Previously infected with tissue-culture material-------	20	2	10.0
	177	42	23.7		20	0	.0
	175	52	29.7				
Mean-------	------	53	29.5	Mean--------	------	1	5.0
Infected with filtered homogenate of victim fish------	196	116	59.2	Previously infected with filtered homogenate of victim fish----------	20	1	5.0
	194	117	60.1		20	1	5.0
	179	102	57.0				
Mean-------	------	111.6	58.6	Mean--------	------	1	5.0
Infected with unfiltered homogenate of victim fish---	192	161	84.0				
	180	154	85.5				
	197	172	87.3				
Mean-------	------	162.3	85.6				

homogenate of victim fish, the so-called "wild" virus, produced 85 percent mortality among previously unexposed 8-week-old fry. The 9-week-old fish (follow-up inoculation of the first experiment) sustained 57 percent mortality; but when they were 13 weeks old, only 15 percent succumbed.

Tissue-culture-propagated virus produced greater mortality among older and presumably more resistant fry in the second trial than it did among younger fry used in the first. The strains of virus were the same, but that which was used in the second trial had been transferred two times less than that which had been used in the first trial. Some pathogens lose virulence when they are separated from their normal host and artificially cultivated for relatively few passages. A reason for the difference in mortality between the first and second trials with the

"laboratory" virus is not known. It may involve some as yet undefined alteration in the virus.

Few if any virus diseases respond to antimicrobial therapy. At present it appears that infectious pancreatic necrosis can be transmitted in the egg. A practical control measure for this disease consists of avoiding sources of eggs which have been associated with high mortality and the whirling behavior in the resulting fry. Where an abundance of eggs is available, extra eggs might be incubated to insure survival of the required number of fish. Though they tend to be the smaller fish, survivors of epizootics of infectious pancreatic necrosis apparently develop in a normal manner and attain maturity. As the eggs are suspected of harboring the agent, it is axiomatic that some adult fish probably act as carriers. Indeed, circumstantial evidence (unpublished) in support of this idea recently has been obtained at the Leetown National Fish Hatchery, Kearneysville, West Virginia.

Summary and Conclusions

Susceptible brook trout were fed a diet which contained tissue-culture-propagated virus of infectious pancreatic necrosis. Coincident with the usual incubation period the fish displayed the behavior of typically infected victims, and fish which died had the usual internal symptoms. Necrosis of the pancreas was identified in the histological examination of such fish. A bacteria-free filtrate prepared from such fish rapidly caused cytopathic change when inoculated into antibiotic-containing tissue cultures. The change was characteristic in appearance and supported a presumptive identification of infectious pancreatic necrosis. The presumptive identification in tissue culture and the specific lesions in the pancreas of victim fish completed the steps of Koch's postulates and established the cause of infectious pancreatic necrosis as being a virus.

Successful infection, and hence confirming evidence, was obtained in a second trial with older fish from the same lot.

Acknowledgment

Mr. George Palmer, superintendent of Maryland's Beaver Creek Hatchery (post office, Thurmont), kindly furnished brook trout eggs for this work.

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A new edition of Prof. Marcel Huet's "Traite de Pisciculture" (Ch. de Wyngaert, 296, Avenue Georges Henri, Bruxelles 15 - Belgique) has recently been published. This third edition includes a revision of the chapters which deal with fish culture in the African tropics and the use of herbicides to control aquatic vegetation, as well as additional bibliographical material.

In less than a year, a tagged brown trout traveled 330 miles and grew 3.5 inches.

EFFECT OF WATER TEMPERATURE AND DIET ON THE BLOOD GLUCOSE OF BROOK TROUT

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A KNOWLEDGE OF THE FACTORS THAT ALTER THE CHEMICAL COMPOSITION OF BLOOD is useful in studies of metabolic rates, food utilization, and physiological processes. The chemical composition of trout blood has been reported to some extent (Phillips et al. 1957, 1958), but the causes of changes in blood chemistry have received little attention. Changes in blood-glucose levels of trout during periods of starvation have been described (Phillips et al. 1954): after 3 days of starvation, blood glucose reached a constant level (about half of normal) and the fish were considered to be in "conservation" metabolism; with continued starvation, the blood glucose resumed a downward trend and, as death neared, reached levels as low as 6 milligrams per 100 cubic centimeters of blood.

The next appropriate step in these studies is the correlation of blood composition with normal fish activity. To this end, a series of experiments was run to measure the effect of diet and water temperature on the blood glucose of trout.

Method

Brook trout (Salvelinus fontinalis) averaging 8 grams in weight were divided into three groups and held in standard hatchery troughs. One group was fed a diet of beef spleen (low carbohydrate); the second, a diet of beef liver, spleen, and dry meals (more than 20 percent carbohydrate); and the third group (the control group) was not fed during the experimental period (temporary starvation).

To assure standard conditions, the trout were fed either the meat or the meat-meal diet for at least 3 days prior to experimental use. The control group of trout, in all experiments, was fed a pre-experimental meat-meal diet. During the experiment, the first two groups were fed at 7 a. m., 11 a. m., and 5 p. m.

A sample of blood was taken just before the 7 a. m. feeding, and a second sample was taken 15 minutes later to determine changes in blood glucose caused by the feeding activity. Additional blood samples were taken at 2-hour intervals throughout a 12-hour experimental period. Blood samples were taken from the control fish at the same time intervals as from the fed fish.

To obtain blood for analysis, a small incision was made through the body wall into the heart. After a pool of blood had collected in the hollow thus made, the blood was drawn into a pipette graduated in 0. 01 milliliters. The blood was transferred to test tubes containing a protein precipitant (1 cc. of 0. 1 N. NaOH and 5 cc. of 0. 45 percent $ZnSO_4$). Blood glucose was determined by the Hagerdorn-Jensen method (1923). This method was rapid and reliable for the small blood samples (0. 02 to 0. 05 cubic centimeter) obtainable from the fish used in the experiments.

For each time interval, three trout were sampled and each experiment was

EFFECT OF WATER TEMPERATURE AND DIET ON THE DAILY FLUCTUATIONS OF BLOOD GLUCOSE OF BROOK TROUT

Hours after initial feeding[1]	First series: Water temperature °F.	First series: Glucose (mg. per 100 cc. of blood) Diet fed[2] A	B	C	Second series: Water temperature °F.	Second series: Glucose (mg. per 100 cc. of blood) Diet fed[2] A	B	C
0.00	47	92	74	92	52.0	93	84	93
0.25	47	86	83	88	52.0	91	89	94
2.00	47	93	91	110	52.4	91	89	106
4.00	47	93	92	113	52.9	93	87	119
6.00	47	93	87	115	55.1	94	91	120
8.00	47	96	81	115	58.4	92	90	120
10.00	47	96	84	115	58.8	97	87	120
12.00	47	97	89	118	59.4	97	93	125

[1] Initial feeding at 7 A.M. Additional feedings at the end of 4 and 8 hours.

[2] Diet composition: Diet A, fish were not fed during the experimental period; Diet B, 98 percent beef spleen, 2 percent salt; Diet C, 25 percent each of beef liver and beef spleen, 12 percent each of wheat shorts, fish meal, cottonseed meal, and distiller's solubles, 2 percent salt.

repeated five times. For each of the experimental groups, a total of 15 analyses was made.

The experiments were run at two water temperatures, one constant at 47° F. and the other ranging from 52° to 59° F. during the 12-hour period.

The diets fed to the fish and the experimental results are summarized in the table. The results are plotted in the chart. Each value in the table and on the chart represents an average of 15 analyses.

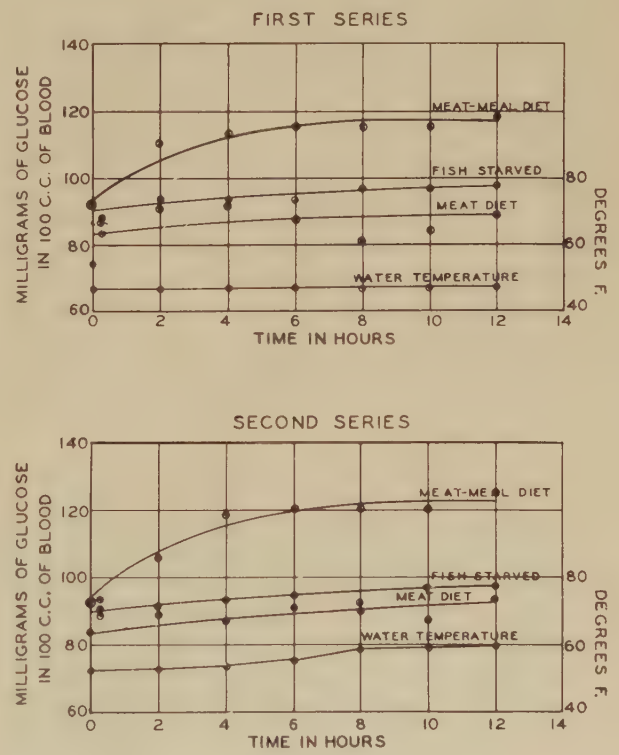
Discussion

Effect of Starvation

Under temporary starvation, no significant changes occurred in the blood glucose of the fish during the 12-hour period at either water temperature. The apparent rise in blood glucose plotted on the chart is not significant.

Effect of Meat Diet

The blood glucose of the meat-fed trout was the same at both water temperatures



A graphic description of the effect of water temperature and diet on the daily fluctuations of the blood glucose of brook trout.

but was significantly lower than the blood glucose of the control fish. The control trout received a meat-meal diet prior to their experimental use, and during the period of experimental starvation they apparently maintained a relatively high level of blood glucose from the previously absorbed diet. Those fish that were fed a meat diet during the experimental period also received a meat diet before the experiments began, and their blood glucose was at a lower level than that of the control fish. The digestion, absorption, and preliminary assimilation of a high-protein, low-carbohydrate diet resulted in a lower level of blood glucose.

Effect of a Meat-Meal Diet

At both water temperatures, the blood glucose of the fish fed a meat-meal diet increased during the experimental period. The increase was highly significant in comparison to the blood glucose of either the control or the meat-fed fish. There is little doubt that the increase resulted from the absorption of carbohydrate from the digestive tract. The fish held in the warmer water had a higher level of blood glucose than those held in the colder water, indicating that digestion and absorption of carbohydrates were more rapid in fish held in the warmer water. Since raw starch was the carbohydrate fed, the rise in blood glucose confirmed the conclusion that trout are able to digest and absorb a considerable amount of raw starch (Phillips, Tunison, and Brockway, 1948).

Effect of Water Temperature

Changes in total metabolism caused by changes in the water temperature had no effect on the blood glucose of the fish. The starved trout had comparable levels of blood glucose at both water temperatures; and the meat-fed fish, although they had less blood glucose than the starved trout, did not differ from each other at the two water temperatures. Only the meat-meal-fed trout showed significant differences in blood glucose at the two water temperatures, and these differences were correlated with the effect of temperature on digestion and absorption, not total metabolism.

Effect of Metabolic Rate

Fish held in warm water had a higher total metabolism than those held in cold water (as evidenced by differences in the growth rates). The increase in total metabolism was not reflected by changes in blood glucose of the trout. If blood glucose of trout had been directly altered by an increase in total metabolism, the blood sugar would have been altered in all fish held in the warmer water.

That some phase of metabolism will alter the blood glucose is shown by the decrease in blood glucose of the meat-fed fish. Although there was no difference in the blood glucose of these fish at the two water temperatures, there was a significant drop at both water temperatures. As the control fish received a high-carbohydrate diet before the experiments were begun, and the meat-fed fish received a low-carbohydrate diet, the difference between the two groups was one of prior feeding and therefore was associated with metabolism of food absorbed prior to the 12-hour experimental period.

Increased activity of the fish during feeding caused an increase in energy utilization and therefore in body metabolism. The blood sample taken 15 minutes after feeding failed to show significant changes in blood glucose that could be attributed to increased physical activity.

In higher animals, the rate of total metabolism is influenced by body activity and the stage of the life cycle. In trout, in addition to these factors, the rate is affected by changes in body temperature resulting from changes in water temperature. The range of blood glucose in man is from 70 to 100 milligrams per 100 cubic centimeters of blood, the actual value depending on diet and body activity. In fish, an increase in metabolism (caused by feeding activity and/or water temperature) did not alter the blood glucose, but a change in a phase of metabolism did (associated with dietary history). Feeding

a meat-meal diet caused an increase in blood sugar that lasted through at least 24 hours of starvation. If, as commonly practiced, the fish had been fed a daily meat-meal ration, the blood sugar would have been maintained continuously at a level considerably higher than that of meat-fed fish. It has not yet been shown whether the high blood-glucose level of meat-meal-fed trout--an occurrence, undoubtedly, in all hatchery trout that are given meat-meal diets containing fairly large amounts of carbohydrates--is desirable.

Phillips, Tunison, and Brockway (1948) reported that trout were unable to regulate the blood-sugar level after a carbohydrate meal in the same manner as the higher animals; these authors described trout as being normally diabetic in terms of human beings. The present studies support these contentions, as the maintained high level of blood glucose indicates a relatively inefficient system for regulating blood-sugar levels.

From these results, it appears that blood glucose cannot be used as a tool to estimate total metabolic activity of the trout body but can be useful as a measure of phases of metabolism. Prior feeding history is important in establishing "normal" values for blood glucose and may be equally important in establishing "normal" values for many of the other blood components.

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BENZENE HEXACHLORIDE vs. LINDANE IN THE CONTROL OF ANCHOR WORM

Benzene hexachloride (BHC) is an insecticide recommended for the control of anchor worm (Lernea sp.) and may be of value in controlling Argulus, Salmonicola, crayfish, and aquatic insects. As marketed in the technical grade, BHC contains a number of isomers, of which gamma is the most active insecticide. The pure gamma isomer is called lindane. Both BHC and lindane are readily available on the market. In calculating dosages, concentrations are usually given in terms of the concentration of pure gamma isomer.

On three occasions I used lindane as a substitute for BHC in treating the golden shiner for anchor worm. The dosage used resulted in the same concentration of gamma isomer as would have resulted from use of BHC. Nevertheless, a heavy mortality of the fish occurred each time.

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THE INFLUENCE OF EXCRETORY SUBSTANCES OF FISHES ON THEIR OWN GROWTH

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THIS WRITER AND HIS COWORKERS reported the chemical analysis of excretory substances of rainbow trout (Salmo gairdneri) that had been placed in a transportation tank (Kawamoto, Tanizaki, and Fujimura, 1957). These workers found that a large amount of nitrogen had accumulated in the water, and that almost all of the nitrogen was present in ammonia. In 1958 this writer also reported that a considerable amount of ammonia was oozing from the bottom soil of a fish farm. Moreover, this writer, with Inouye and Nakanishi (1957), studied the relation between the pond area, densities of fish, and the growth rate of carp (Cyprinus carpio).

Unfortunately, why the fish in populations of large density grow less well than fish in populations of small density has not yet been determined (Kawajiri 1928, Kawajiri and Hata 1935). Studies of the present writer and coworkers (Kawamoto, Inouye, and Nakanishi, 1957), however, made it clear that such inhibition of fish growth came mainly from the accumulation of large quantities of excretions such as ammonia, and that the presence of phytoplankton and algae in the water affected fish growth because the availability of ammonia varied with carbon assimilation.

Brockway (1950) studied the effect of excretory ammonia on the growth of trout; he reported that they died when the ammonia level reached 0.3 p.p.m., and that this phenomenon was caused by the effect of rising ammonia concentrations on the ability of the fish to use oxygen.

The present writer has conducted quantitative experiments to determine how the growth of carp is affected by accumulations of metabolic wastes.

Materials and Methods

For the experiment on the effect of ammonia on the growth of fish, 10 young carp were employed. A colored bead was attached in front of the dorsal fin of each fish with enameled wire (table 1). The

TABLE 1.--Initial weights and lengths of 10 marked carp placed in 2 aquariums

Color of bead on carp	Carp in aquarium G[1] Weight (grams)	Carp in aquarium G[1] Length (centimeters)	Carp in aquarium H[2] Weight (grams)	Carp in aquarium H[2] Length (centimeters)
White (W)-------	2.7	4.8	3.5	5.1
Green (G)-------	3.1	4.8	2.7	4.7
Blue (B)--------	2.5	4.8	2.1	4.3
Dark blue (DB)--	2.6	4.7	3.0	5.0
Red (R)---------	2.8	4.9	2.7	4.7
Average------	2.74	4.8	2.80	4.76

[1] Five liters of water.

[2] Five liters of 0.3 p.p.m. solution of ammonium chloride.

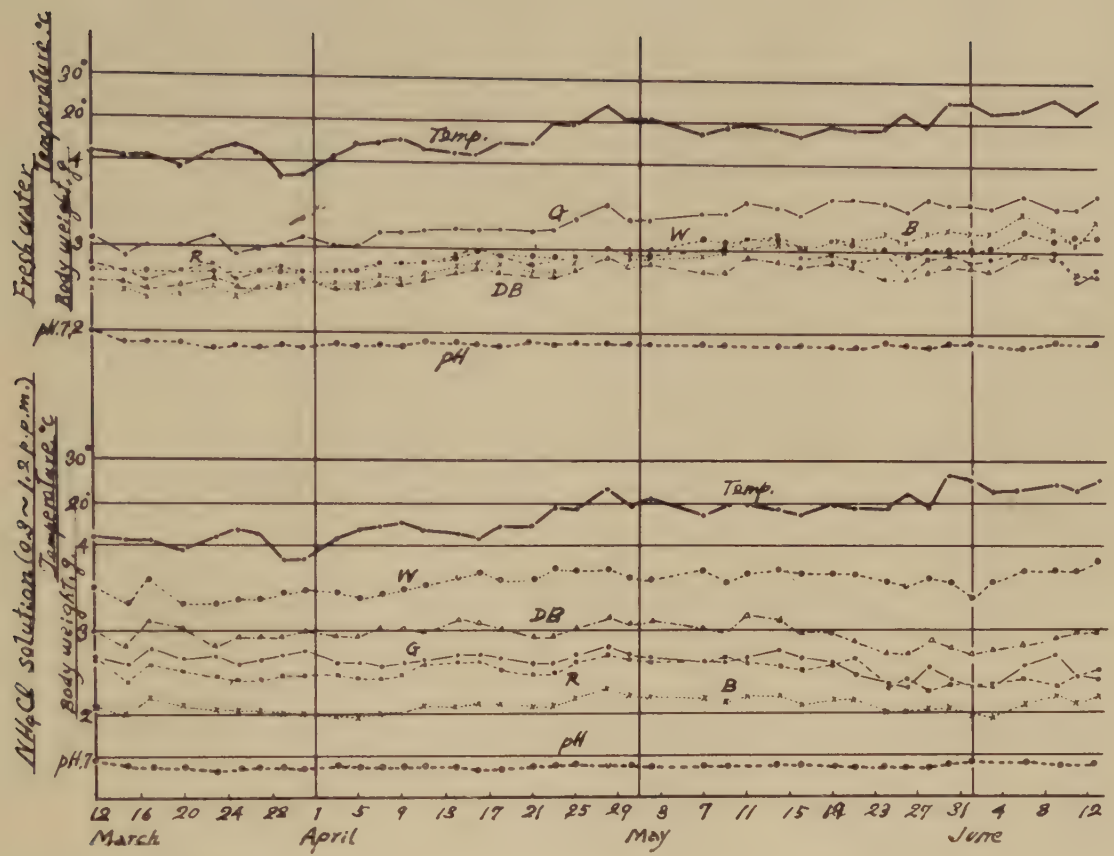

FIGURE 1.--The influence of ammonium chloride solutions on the growth of carp.

carp were divided into two groups. One group was put into a glass aquarium containing 5 liters of water, and the other was put into a glass aquarium containing 5 liters of 0.3 p.p.m. solution of ammonium chloride. Every 2 or 3 days, each carp was weighed precisely and both the water and the solution were renewed so as to remove the influence of accumulated excretory substances. Once a day, wriggler worms (mosquito larvae) were given to the fish as a food. It should be noted that the arrangements were made so that not a bit of food was left over when the fish stopped eating, and all conditions were maintained as stable as possible.

Experiment 1. Ammonia and Growth of Carp

To make clear the influence of ammonia on fish growth, the writer compared the growth of carp reared in normal water with the growth of carp reared in solutions of ammonium chloride from March 12 to June 13, 1959.

Table 2 shows that the average weight of the carp in the aquarium containing the normal water increased from 2.74 grams to 3.13 grams during the 91-day period, while in the aquarium containing the solutions of ammonium chloride this average weight decreased from 2.80 grams to 2.77 grams. (See also figure 1.)

TABLE 2.--Weights of 10 marked carp held in normal water and in ammonium chloride for 91 days

	March		April			May				June	
	12	23	3	11	21	2	11	21	30	9	13
						(grams)					
Aquarium G[1]:											
pH[2]	----	6.25	6.4	6.5	6.5	6.4	6.4	6.4	6.58	6.72	6.70
Water temperature, °C.[2]	----	12.0	10.8	12.7	14.0	20.5	19.2	18.5	25.0	25.8	25.9
Carp (W)	2.70	2.70	2.70	2.80	2.88	2.98	3.10	3.05	3.00	3.10	3.15
(G)	3.10	3.10	3.00	3.17	3.18	3.35	3.55	3.60	3.55	3.50	3.65
(B)	2.50	2.55	2.55	2.70	2.69	2.89	2.90	2.80	2.80	2.95	2.70
(DB)	2.60	2.65	2.50	2.60	2.65	2.80	3.10	3.10	3.25	3.25	3.35
(R)	2.80	2.80	2.70	2.80	2.80	2.90	3.00	2.85	2.95	2.95	2.80
Average	2.74	2.76	2.69	2.81	2.84	2.98	3.13	3.08	3.11	3.15	3.13
Aquarium H[3]:											
pH[2]	----	6.25	6.5	6.5	6.5	6.5	6.4	6.4	6.60	6.50	6.60
Water temperature, °C.[2]	----	12.0	10.8	12.4	14.0	20.8	19.2	18.5	27.0	24.8	25.0
Carp (W)	3.50	3.30	3.40	3.50	3.55	3.58	2.20	2.15	2.05	2.20	2.20
(G)	2.70	2.70	2.60	2.63	2.59	2.65	3.65	3.65	3.55	3.70	3.80
(B)	2.10	2.05	1.55	2.08	2.04	2.18	2.60	2.65	2.35	2.30	2.50
(DB)	3.00	2.80	2.90	2.97	2.90	3.07	2.65	2.45	2.40	2.70	2.40
(R)	2.70	2.45	2.45	2.56	2.46	2.60	3.15	2.85	2.80	2.90	2.95
Average	2.80	2.66	2.66	2.74	2.71	2.81	2.85	2.75	2.63	2.76	2.77

[1] Five liters of water.

[2] Measured at noon.

[3] Five liters of 0.3 p.p.m. solution of ammonium chloride until the 58th day; thereafter, up to 1.2 p.p.m.

This experiment proved clearly that the growth of carp, like that of the trout reported by Brockway (1950), was disturbed by the presence of ammonia. It was found, moreover, that the resistance of the carp to the ammonia was greater than that of trout, as a solution of 0.3 p.p.m. ammonium chloride was fatal to the trout, whereas the carp survived even in 1.2 p.p.m. in spite of the decreasing body weight. It can be inferred, nevertheless, that the carp will die when there is too great an accumulation of excretory substances in the water.

Experiment 2. Ammonia and Oxygen Consumption of Carp

To examine the cause of the inhibition of growth, the writer compared the oxygen consumption of carp reared in normal water with the oxygen consumption of carp reared in the 1.2 p.p.m. solution of ammonium chloride. For this experiment the difference in oxygen content in the inlet and outlet waters of the chamber containing a carp was measured by the micro-Winkler method.

Table 3 shows that the effect of the solutions of ammonium chloride on the oxygen consumption of the fish decreased at low temperatures and increased at high temperatures.

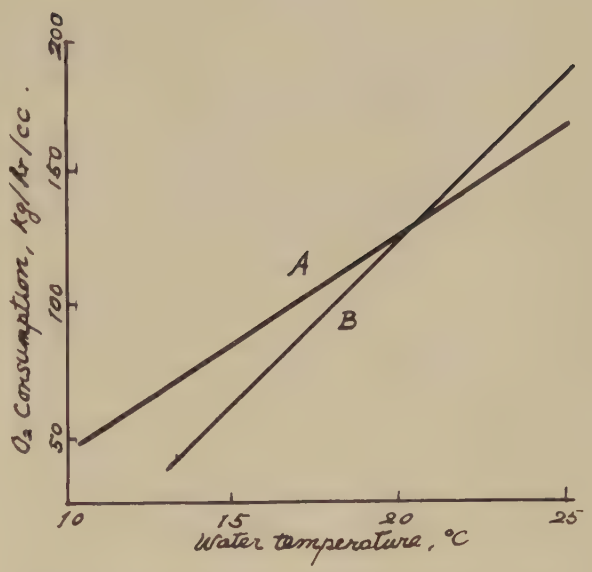

FIGURE 2.--Comparison of oxygen consumption by carp in the normal water and by carp in the 1.2 p.p.m. solution of ammonium chloride. A -- Normal. B -- 1.2 p.p.m. of ammonium chloride.

TABLE 3.--Oxygen consumption of carp held in normal water and in 1.2 p.p.m. solution of ammonium chloride for 10 days

Normal water[1]			1.2 p.p.m. of ammonium chloride[1]		
Water temperature °C.	Body weight (grams)	Oxygen per kg./hr./cc.	Water temperature °C.	Body weight (grams)	Oxygen per kg./hr./cc.
25	57.6	177.3	25.5	57.5	197.1
25	57.6	178.0	26	57.5	186.3
24.8	8.1	224.0	25.5	6.8	190.7
25	31.4	231.6	25.2	36.2	227.1
21	33.9	133.2	20.8	36.9	162.5
21	33.9	120.1	21	35.7	114.3
21	33.9	130.6	21	35.7	113.6
13.5	60.2	46.0	13.2	76.5	43.2
13.5	60.2	43.2	13.2	76.5	37.9
13.5	60.2	45.8	13.2	76.5	37.4

[1] Ten liters.

Using the data in table 3, the writer calculated the least square in the limit of temperature from 13.5° to 25° C. and got the following equations:

In normal water,

$$Y = 8.2x - 33.2.$$

In 1.2 p.p.m. solution of ammonium chloride,

$$Y = 13x - 130.8.$$

These equations are shown in figure 2.

Experiment 3. Feces of Fish

Some papers have dealt with the effect of fish urine on their activity, but no study of

the effect of fish feces has been made. Of the opinion that the feces should play as important a role as urine in the fish farm, the writer made the following study with young goldfish (Carassius auratus) for the purpose of clarifying this subject.

The goldfish were reared in a white-tiled pond 2 square meters in area, 1/3 meter in depth. They were given food mixed with meal of silkworm pupae, wheat flour, and rice bran once a day. An adequate amount of tap water was permitted to flow into the pond. An effort was made to have the water run off the surface during the whole 2-month period of the study.

At 2 p.m. on August 14, when the experiment was begun, the goldfish weighed 2,516 grams, the temperature of the water was 29° C., and the pH was 8.05. At 2 p.m. on October 15, when the experiment was terminated, these fish weighed 2,940 grams, the temperature of the water was 20.5° C., and the pH was 8.34. During this period all the fish had been swimming vigorously.

As the bottom of the pond was white tile, the deposit of feces and almost any phytoplankton or algae suspended in the water could be clearly observed. On October 15, when about 80 percent of the bottom was covered with feces, almost all of the accumulation was taken up with the siphon.

The feces collected naturally contained some dust and other matter, but almost all were a few millimeters in length and dark green in color. To remove water, the feces were filtered and spread over filter papers at room temperature. The half-dried feces weighed about 600 grams at room temperature.

As the fecal material was dark green, the writer examined it under the microscope. He found pieces of capsule of silkworm pupae, protozoa (especially vorticella and nematoda), and microalgae--Phormidium sp., Scenedesmus obliquns, Oocystis parva, Pediastrum araneosum, Flagellata (in cyst), and Synedra sp.--attached to the feces.

As the half-dried feces contained 18 percent water, the dry substance weighed 492 grams. The total nitrogen in the dry matter, determined by the Kjeldahl method, is shown in table 4.

The 5.78 percent nitrogen of the dry matter (table 4) included that of microalgae, protozoa, and other material. It should not be overlooked, however, that a considerable amount of the nitrogen did exist in the dried feces. Such nitrogen compounds will be changed into ammonia by the decomposition of the feces, and then nitrate and nitrite will appear gradually--through the action of bacteria--and will also affect the growth of fish.

TABLE 4.--Total nitrogen content in the dried feces

Sample number	Weight of sample (grams)	Total nitrogen (percent)
1	0.100	5.356
2	0.070	6.451
3	0.098	5.501
4	0.088	5.774
Average		5.780

Discussion

In Experiment 1, the writer determined quantitatively the influence of ammonia on the growth of the carp, finding that the carp had more ability than the trout to resist the effects of ammonia.

In Experiment 2, the writer compared the respiration of fish in the ammonia solutions with their respiration in normal water, finding that the oxygen consumption of the carp which were reared in 1.2 p.p.m. solution of ammonia decreased at low water temperatures but increased at high temperatures. From these results, the writer considered that the change in oxygen consumption might indicate the influence of the oxygen dissociation curve of the blood, as Brockway stated (1950). The carp were alive in the 1.2 p.p.m. solution during the experiment, but it can be supposed that the fish would die of the effect on respiration when the ammonia reached a higher concentration.

In Experiment 3, this writer studied the feces of goldfish (total weight, 2,516 grams) and found the amount to be unexpectedly large--about 492 grams of dried feces within 2 months. The dried feces of the goldfish contained 5.78 percent nitrogen (24.4 grams), whereas Gerking (1952) reported 7.27 percent with a standard deviation of ± 1.08 for sunfish.

Gerking (1955) reported that sunfish having a total weight of 29.7 grams might be expected to excrete 7.18 milligrams of nitrogen in the urine per day. Calculated on the basis of Gerking's work, the amount of ammonia excreted in the urine of goldfish whose total weight is 2,516 grams will be 608.2 milligrams per day and will reach 36.49 grams within 2 months. However, part of this ammonia of the urine will pass through the outlet and have no effect on the fish. The amount of ammonia remaining--from urine and decomposed fecal substances--is very large, but all of it does not always affect fish growth, because microalgae attached to the feces or phytoplankton will absorb some of it. The nitrogen content of the water decreases during the day but increases during the night. Thus a large part of the excreted nitrogen accumulated during the night in the stagnant pond will affect fish growth (Kawamoto, Inouye, and Nakanishi, 1957). In running-water ponds, the number of the phytoplankton or microalgae is very small, and their effect may be overlooked except when few algae or weeds grow on the bottom of the pond. Therefore, when many fish farms are located along the same stream, the nitrogen in the water increases proportionately in the ponds farther downstream, and the fish in such ponds will be more affected by the ammonia than fish in the pond located upstream, as reported by Brockway (1950).

Conclusion

The excretions of fish, whether urine or feces, largely inhibit the growth of fish. The presence of phytoplankton or algae is closely related to the effect of excretory substances on the fish. Therefore, fish-culturists should never disregard the accumulation of the excretory products of fish--especially in the stagnant pond--and should always endeavor to remove the influence of these products.

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THE FATE OF KIDNEY DISEASE AMONG HATCHERY BROOK TROUT STOCKED IN NATURAL WATERS

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KIDNEY DISEASE is a bacterial infection of salmonid fishes that has been reported from hatcheries located in widely scattered areas of the United States. 1/ Mortality from the disease may be high.

A number of studies concerning the effect of the disease on fish and its control at hatcheries have been conducted (Earp, Ellis, and Ordal, 1953; Wood and Wallis 1955; Snieszko and Griffin 1955; Allison 1958; and Wolf and Dunbar 1959a, 1959b). Snieszko and Griffin (1955) reported that brook trout exposed to infection through contact or by the feeding of infectious material did not develop the infection during an observation period lasting several months. They succeeded in inducing the disease in healthy trout only by intraperitoneal inoculation with virulent material. Because of this, they suggested that a parasitic vector, such as a gyrodactylid trematode, might be involved in the transmission of kidney disease. These observations suggest that an abundance of the parasites must be present to effect the transmission of the disease from infected hatchery fish stocked in lakes or streams to fish already there. Wolf and Dunbar (1959b) were unable to infect fish by the addition of the bacterium or the bacterium and glass splinters to the diet, but they did induce the disease by means of manual abrasion and autoabrasion in the presence of the bacterium. Thus certain hatchery conditions, such as the crowding of fish into hard-bottomed raceways, would be conducive to transmission of kidney disease. Because trout in natural waters are seldom so crowded as trout in hatchery ponds and are rarely heavily infested with parasites, it would seem that the possibility of producing an epidemic among salmonids in natural waters by stocking fish infected with kidney disease would be remote. Dr. Ken Wolf reported (in a personal communication) that kidney disease was diagnosed among brook trout in a stream near the National Fish Hatchery at Berlin, New Hampshire; but published references to kidney disease in nature are concerned only with various species of salmon. In the literature, no information could be found concerning the fate of infected hatchery trout when stocked in lakes or streams, or whether the disease was transmitted from these fish to other trout inhabiting natural waters. The work described here was designed to investigate these questions.

Method

In 1955 and 1956, at the Oden hatchery in the Lower Peninsula of Michigan and at the Marquette hatchery in the Upper Peninsula, there were brook trout (Salvelinus fontinalis) infected with kidney disease. Presence of the infection was confirmed by autopsy of fish that died, and mortality

1/ Wolf, Ken. Survey on the occurrence of kidney disease in trout hatcheries in some inland states. Processed. Bureau of Sport Fisheries and Wildlife, Eastern Fish Disease Laboratory, Leetown (post office, Kearneysville), West Virginia. 1956.

in both groups was well above that among similar groups of brook trout that were free of kidney disease.

Fish from the infected group at the Oden hatchery were stocked in a number of lakes in the Oden district. Two small lakes chosen for study, North and South Twin Lakes, Otsego County, are located within the area of the Pigeon River Trout Research Station and therefore under the observation of the staff. Here, under the provisions of the angler's permit, all fish caught from any waters of the research area must be weighed, measured, and sexed at a checking station. Thus all fish are observed by a member of the staff, and any symptoms of kidney disease could be noted. Also, fish stocked in the lakes are fin-clipped with various combinations to indicate the year of stocking. If infection were transmitted to fish stocked in previous years, this fact could be recorded at the checking station.

Fish from the infected group of trout at the Marquette hatchery were stocked in certain streams and pothole lakes; and collections to be checked for kidney disease were made several months later, or early the following year, by netting, electric shocking, and poisoning.

Swanzy Lake, Marquette County, was studied because of its accessibility and the ease with which fish could be collected. As part of the management program, fish stocked there are fin-clipped according to the year released; so, if kidney disease did spread from introduced infected fish, newly infected fish could be identified. Although the lake is on public property and is open to fishing without special permit, it was on the regular schedule of a creel-census clerk who was able to observe many of the fish caught, as well as any fish mortality that might have occurred.

Conservation officers of the Marquette area learned the symptoms of kidney disease by observing infected fish at the Marquette hatchery so that they would be able to recognize affected fish when inspecting anglers' catches or observing possible fish mortalities in waters that had been stocked with trout from the infected group.

Results

Brook trout from the infected groups held at the Oden and Marquette hatcheries were stocked in 25 lakes during the fall of 1955 and 1956. Three of these lakes were checked during the following spring for fish with symptoms of kidney disease.

On April 14, 1956, 37 brook trout were collected from Swanzy Lake. All had been stocked in the fall of 1955, and symptoms of kidney disease were present in three specimens at the time of capture. Another collection was made on October 10, 1956. This time 18 fish (of which 9 had been stocked in 1954 and 9 in 1955) were taken. Of the 18 fish, 1 (stocked in 1955) had symptoms of the disease, but the rest did not. Neither the creel-census clerk nor the conservation officers reported any affected fish or any mortality of fish from any location where infected brook trout had been stocked.

At the Pigeon River Trout Research Station, 17 brook trout were collected on May 31, 1956, from North Twin Lake. Seven of these were from the infected group stocked in the fall of 1955, nine had been stocked in the fall of 1954, and one had been stocked in the fall of 1953. There were no symptoms of kidney disease among these fish. A number of 7- to 10-inch brook trout from the infected group held at the Oden hatchery had been stocked in South Twin Lake in the fall of 1956. In May 1957, five fish (four stocked in 1956 and one in 1955) were collected. All were negative for kidney disease. In November 1957, 23 fish (14 from the 1955 stocking and 9 from the 1956 stocking) were collected here. None showed symptoms of kidney disease. None of the fish caught by anglers and checked at the station were observed to have this disease, and neither anglers nor staff members observed any mortality of fish in either lake.

Fish from an infected group of brook trout were stocked in the spring of 1956 in the Peshekee River, the Chocolay River, and the East Branch of the Escanaba River--all in Marquette County, Michigan. On October 22, 1956, a survey crew of the

Institute for Fisheries Research collected 43 brook trout and 8 brown trout from the Peshekee River. None of these fish had any symptoms of kidney disease. In June 1957, 21 brook trout were collected from the Chocolay River and the East Branch of the Escanaba River. None of these trout were infected with the disease. No fish mortality was seen by the conservation officers who patrolled the streams, and none was reported by any of the anglers whom they contacted.

Conclusion

In all, 164 trout were collected from natural waters and examined for kidney disease. There were no instances in which the disease was transmitted from stocked fish to fish already in the waters. No mortalities of trout were reported from any of the lakes or streams which were stocked with brook trout infected with kidney disease, although some of the waters were regularly visited by personnel of the Department of Conservation. Therefore, it appears unlikely that the stocking of infected brook trout in lakes and streams can result in a widespread mortality due to kidney disease.

Acknowledgments

The assistance of Institute for Fisheries Research survey crews and personnel at the Marquette and Pigeon River Research Stations in collecting fish from various locations is gratefully acknowledged. The excellent cooperation of personnel at the Oden and Marquette hatcheries is appreciated.

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Most species of trout and salmon which spend long periods in fresh water can be reared on dry feeds, but chinook salmon cannot. At the Western Fish Nutrition Laboratory (Cook, Washington) of the Bureau of Sport Fisheries and Wildlife, experiments which involved duplicate lots of chinook fry that were fed diets of dry commercial fish feed moistened with different levels of water revealed that 30 to 35 percent moisture content is necessary for maximum growth. This result supports the hypothesis that chinook salmon cannot voluntarily ingest water or otherwise moisten ingested dry feed.

ICHTHYOPHTHIRIASIS IN A RECIRCULATING CLOSED-WATER HATCHERY

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AN OUTBREAK OF THE CILIATE PROTOZOAN Ichthyophthirius multifiliis Fouquet infested 30,000 steelhead fingerlings (Salmo gairdneri) in the Humboldt State College hatchery on July 16, 1958. The disease soon spread throughout the station, infesting 50,000 silver salmon (Oncorhynchus kisutch) fingerlings maintained in two 100-foot-long concrete raceways. The station's yearling rainbow (Salmo gairdneri) and cutthroat (S. clarki clarki) brood stocks also served as hosts to the parasites but to a considerably less extent than the fingerlings. Condition of the fish was not considered as a primary factor in the cause of infestation. Prior to the outbreak, each lot of fish had incurred an insignificant mortality and was progressing satisfactorily.

The Humboldt State College hatchery is a unique station in that it utilizes a recirculating closed-water system (DeWitt and Salo 1960). Water, after leaving the ponds and troughs, is collected by gravity flow into a primary sump; it is then pumped through a set of three 72-inch-diameter sand-and-gravel filters and an aerator. From the aerator the water is returned by gravity flow to the ponds and troughs, thus completing the cycle.

Source and Spread of the Disease

Water for the station's recirculating system is obtained from a small, spring-fed creek which is completely void of fish life. Because of these characteristics, the probability that the disease was introduced via the water supply has been discounted.

The hatchery has been the recipient of lots of fingerling and yearling salmonids transferred from stations which have been known to contain Ichthyophthirius. As each individual lot of fish was delivered, samples were taken and superficially surveyed for external parasites. All observations proved negative.

Fish arriving at the station are placed directly into the outdoor circular ponds and raceways. The desirable practice of isolating questionable shipments of fish for a given period of quarantine is not applicable with a station of this type. It is therefore highly probable that the disease was introduced by a transferred lot of fish that had either a latent infection (Schaeperclaus 1933, 1954) or one that was active and had gone undetected.

The first appearance of the disease was noticed among the steelhead which had been hatched and reared to a fingerling stage (58 per ounce) in the college hatchery. All fish-production implements are strictly confined to their specific areas of use; however, for additional safety, the implements confined to the hatchery were immersed in a 25-percent sodium chloride solution after being used in or around the infected area. It was assumed that the three sand-and-gravel filters would prevent the spreading of the disease from the hatchery troughs to the outdoor ponds and raceways. As the infected fingerlings were held in troughs, the conventional fast-water-flow treatment

of removing the parasites as they leave the host was the only method employed.

Within 2 days considerable flashing and erratic rubbing against the sides and the bottom of the raceway were noted among the silver salmon, and to a lesser extent flashing was also evident among the station's yearling brood stock. It was surmised that the filters were allowing a stage or stages of the life cycle of Ichthyophthirius to pass through 2.5 feet of filter sand.

Inspection of each of the filters revealed them to be in good condition and functioning properly. The three units are operated at or slightly above their normally rated output of 3 gallons per minute per square foot of filter bed area. At this rate, the possibility that some individuals are not removed by filtration has been confirmed by D'Elia. [1/]

Although the outbreak of the disease was first noted in the fish in the hatchery, it is reasonable to assume that the parasites had originated and progressed undetected among the older fish in the outdoor ponds.

Temperature and Rate of Maturation and Encystment

During the period of the epidemic, the temperature of the recirculating water ranged between 62° and 66° F. At these temperatures, the parasites reached maturity and dropped from the host in 8 to 10 days. The total time required for encystment, completion of divisions, and emergence of young ciliospores from the cyst in a constant temperature of 66° F. varied greatly among individual parasites. The earliest observed emergence of ciliospores occurred within 12.5 hours from the time the parasite dropped from the host, whereas the longest period recorded was 52 hours. MacLennan (1937) reported that the variations were partly induced by the age differences of the individuals upon leaving the host; apparently the efficiency of encystment increases with the length of time the parasite remains on the host. MacLennan also inferred that extreme variations, even among individuals of the same age group, occurred between the time of completion of divisions and the eventual emergence of ciliospores from the cyst.

Conditions of Treatment

The literature indicates that difficulty in attempts to control this disease by chemotherapy is correlated with an insufficient number of applications.

In the course of this study, no chemicals were found which would destroy Ichthyophthirius during the parasitic stage. The ability of the ciliospores to burrow through and eventually embed themselves beneath the epidermal layer of the host (Wolf 1938) apparently affords them adequate protection from external therapy (figures 1, 2).

The exposed, free-swimming and encysted parasites were easily destroyed by

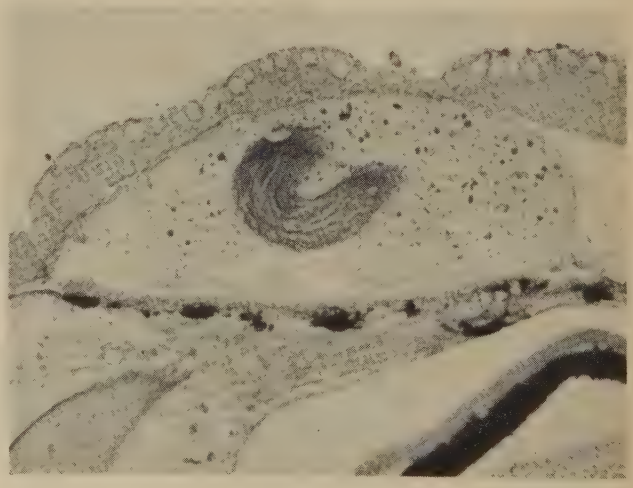

FIGURE 1.--Longitudinal section of a steelhead fingerling showing a large individual with distinct horseshoe-shaped macronucleus. The remnant of epidermal cells at posterior end of parasite has been dislodged and broken into small globules for ingestion by the individual's powerful ciliary beating activity. X 200.

[1/] Personal communication from Mr. R. A. D'Elia, manager of the Garratt Engineering and Equipment Company, Millbrae, California.

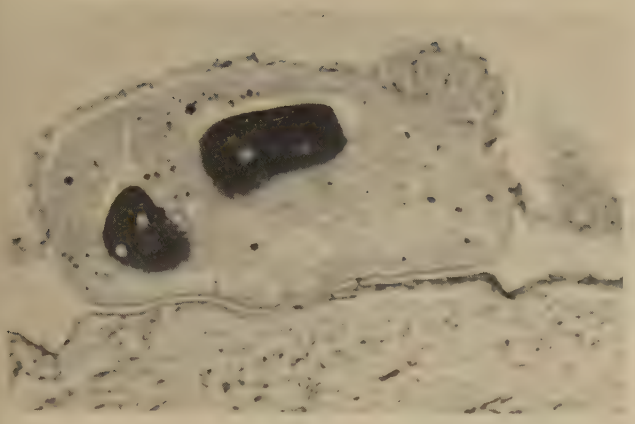

FIGURE 2.--Cross-section of a silver salmon showing an advanced individual which has formed an epidermal blister prior to leaving its host. Note hyperplasia and and separation of epidermis from the deeper layers of tissue. X 200.

chemicals; however, therapy was complicated by the variable periods at which the mature parasites left the host and by the possibility that some individuals were capable of procreating infective ciliospores within 12.5 hours. It was apparent that efficient control would require the application of a minimum of two treatments per day for a period slightly longer than the duration of the parasitic stage.

Though a continuous chemical treatment is mechanically possible with a station of this type, the severity of the attack had weakened the fish to the extent that experimentation with this specific application was not advisable.

Treatment

Exposure of the weakened fish to numerous treatments required the selection of an economical chemotherapeutic agent that could be administered frequently with comparative safety. As a result of experiments with various chemicals and their combinations, a solution of 0.25 percent salt and 1:800,000 malachite green (chloride) was found to display the desired qualities. The toxicity of various concentrations of a salt and malachite green solution on Ichthyophthirius is shown in the table.

For ease of application, the desired amounts of salt and malachite green were dissolved and mixed in containers with pond water. The treatments were administered by the prolonged bath method (Fish 1947) for durations of 30 minutes; however, the general practice of lowering the water to a desired level prior to administering the solution was avoided. The cysts of Ichthyophthirius possess an adhesive quality which may have enabled some of the individuals to remain attached above a lowered level of treatment. Although the cysts are not resistant to dessication (Butcher 1947), it was assumed that because of the short duration of treatments the exposed surfaces of ponds, especially the earthen brood pond, would not have sufficient time to dry thoroughly.

At the conclusion of a treatment, the chemical solution was flushed from each of the treated ponds by restoring the water flow and gradually opening the outlet gates. The flushed solution was allowed to enter the primary sump, where the

THE TOXICITY OF SALT AND MALACHITE GREEN ON THE ADULT FREE-SWIMMING AND ENCYSTED STAGES OF Ichthyophthirius AT 66° F.[1/]

Concentration of solution		Average time required to destroy the parasites	
Percentage of salt	Malachite green	Free-swimming stage	Cyst stage
		(minutes, seconds)	
1.5	1:15,000	Instantaneous	1.--
0.5	1:60,000	4.03	8.--
0.25	1:200,000	9.36	14.--
0.25	1:400,000	12.28	17.--
0.25	1:800,000	18.00	25.--

[1/] A series of 10 tests, using one parasite per test, was performed for each stage at the various concentrations. Each test was terminated after the parasite's revolving endoplasmic movement and ciliary beat had ceased.

greater percentage overflowed and was removed from the system via the sewer. The dilute portion of the solution which remained in the sump was pumped through the filters, where additional chemical agent was removed. Subsequent dilution of the filtrate resulted in an insignificant concentration of the solution in the system. The accumulation of chemical agents in the system was prevented by cleaning the ponds and back-flushing the filters between each of the successive treatments.

The treatments were administered two times a day for 11 days. During this period there was no loss among the yearling rainbow and cutthroat brood stocks. The high mortality which the heavily infested lots of steelhead and silver salmon fingerlings had been suffering decreased and became negligible in the latter stages of treatment. The absence of a significant increase in fingerling mortality during and after the period of therapy suggests that no lethal cumulative toxicity resulted from the successive treatments.

By the eighth day of treatment, sufficient improvement had resulted from this therapy; however, it was only through the required number of successive applications that reinfestation was prevented and the epidemic was eventually brought under control.

Acknowledgments

Grateful acknowledgment is extended to Mr. J. W. DeWitt and Dr. E. O. Salo of Humboldt State College for their editorial assistance, and to Mr. Harold Wolf, fisheries pathologist, California Department of Fish and Game, for his review of this manuscript.

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Experiments at the Eastern Fish Nutrition Laboratory (Cortland, New York) of the Bureau of Sport Fisheries and Wildlife were designed to evaluate the practice of grading hatchery trout by size. If fish of a particular size are specified in a management program, grading may be desirable and necessary. Results of the experiments in terms of growth rates, however, show that it is doubtful that cost of grading may be regained if the sorting is done in an attempt to increase hatchery production and feeding efficiency.

RESEARCH UNITS FOR EGG INCUBATION AND FINGERLING REARING IN FISH HATCHERIES AND LABORATORIES

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IN THE PAST WHEN ENVIRONMENTAL CONTROL was needed for experiments in hatcheries, it was necessary to maintain separate lots of fish eggs, fry, or fingerlings in individual tanks or troughs with separate water supplies. Generally these holding units were from 6 to 12 feet long and 18 to 36 inches wide. In laboratories, aquariums were used. Therefore, the experiments were greatly restricted because of the space needed for the containing units.

Experiments in genetics, nutrition, fungicides, radiation, disease, and other fields can usually be accelerated when a greater number of identical environments is available. Methods for increasing the number of similar environments while reducing the amount of space and water required will be discussed in this paper.

Description and Operation of Units

A method for incubating experimental lots of eggs was developed from a modification of the jar culture of trout eggs (Buss 1959a, 1959b). This unit consists of a battery of laboratory polyethylene bottles, either pint or quart size. The water is supplied through a 2-inch galvanized pipe. One-half-inch pipes lead from the main 2-inch pipe to a tee. A standpipe is inserted on the upper end of the tee. On the lower end of the tee, a rubber stopper drilled for a section of 1/8-inch (inside diameter) rigid plastic tubing is inserted. This plastic tubing conveys the water flow for each bottle. The bottom is cut out of the bottle; and the bottle is inverted and screwed into its cap, which has been fastened in a drainage channel. The overflow of water can be passed either through small holes drilled near the upper surface of the bottle or through fine-mesh galvanized hardware cloth which has been fastened to the inside of the container. The hardware cloth can be attached by softening the polyethylene with a hot soldering iron and then pressing the cloth into the soft plastic. A unit comprising 202 quart bottles in three tiers of two rows each measures 12 feet 6 inches long, 2 feet 3 inches wide, and 3 feet 10 inches high. (See figure 1.) A unit with the same number of pint jars can be made more compact.

The polyethylene bottles are set up for egg incubation by placing a tight-fitting stopper in the neck of the bottle. Gravel (1/4-inch) is placed on top of the stopper to fill the curvature of the bottle. The stopper prevents the gravel and eggs from falling out when the bottle is removed. Hardware cloth is then placed on top of the gravel to prevent the eggs from falling into the gravel. The 1/8-inch tubing is inserted through a hole in the screen to within 1/4 inch of the stopper. This tubing disperses the water evenly through the gravel and hence through the eggs. (See figure 2.)

As many trout eggs as the bottle will hold have been successfully eyed and hatched. After the eggs hatch, the dead eggs can be removed by pouring the eggs and fry on a hatching screen which allows

FIGURE 1.--Research unit for incubating trout eggs in similar environments. The insert is a close-up view of polyethylene bottles containing incubating eggs.

the sac fry to fall through and retains the dead eggs. The sac fry can then be placed back in the bottle until they swim up. (See figure 3.) During the incubation of the eggs and the development of the sac fry, the unit should be treated daily with a 1:600 concentration of formaldehyde for 15 minutes.[1/] A constant-flow siphon can be installed on a standpipe at the head of the unit for treatment. After the fry swim up, if the lot is to be kept, they can be transferred to large jars.

The polyethylene-bottle unit uses about 15.5 gallons of water per minute for 202 bottles. Each bottle receives about 0.3 quart per minute.

Trout fingerlings can be reared in the larger jars (6.5 to 7 quarts) used to incubate production lots of trout eggs (Buss 1959a, 1959b). The only modification necessary is to remove the gravel and wire mesh and screen off the overflow. The top can be screened with hardware cloth that is held tight to the jar by a tension device. Wire loops or short sections

[1/] Reddecliff, J. M. Formalin as a fungicide in the jar method of egg incubation. Notes for Fish Culturist from the Benner Spring Fish Research Station, 1958.

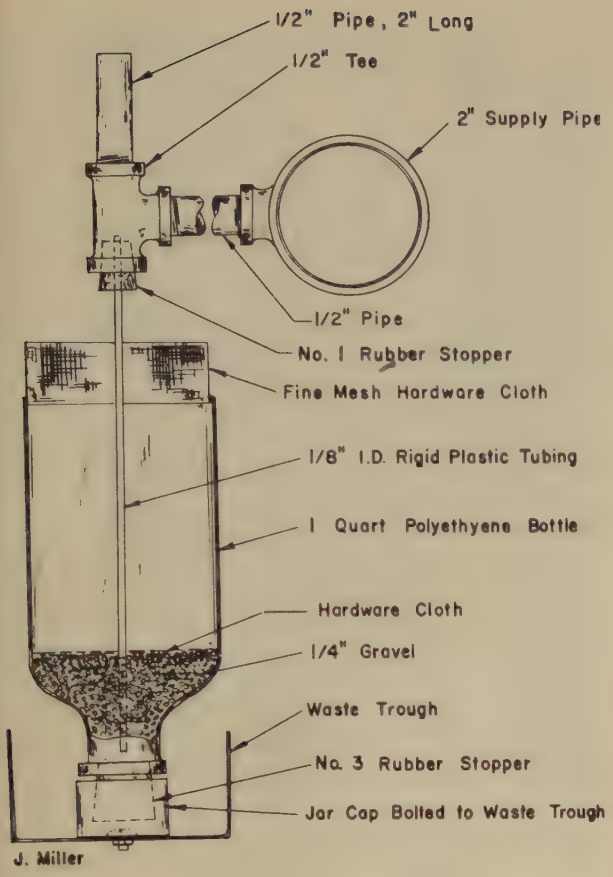

FIGURE 2.--Schematic drawing of research unit with setup of polyethylene bottle.

of rubber garden hose spliced with a dowel pin can be fashioned to fit the inside diameter of the jar and hold the screen tight against the surface of the jar. Trout fingerlings feed readily in these jars because the feed is in constant motion. The jars are partly self-cleaning, and daily cleaning is not necessary if the fish are fed properly. At the Benner Spring Station the jars are cleaned once a week by emptying each jar into a tub and then replacing the fish in the jar. If necessary, the fingerlings can be treated twice weekly with formaldehyde for external parasites and fungus.

The jars are usually started with 300 swim-up fish. In about 90 days, when the fingerlings reach a length of 2 inches, the number is reduced to 100. These fish do well until the lots weigh 1 to 2 pounds. Trout have been held in these jars for a year, but their number was greatly reduced. The limiting factor seems to be space, as mortalities of larger fish are caused by the pinning of some fish to the bottom of the jar by the bulk of the other fish present. Flow in the larger jars is maintained at about 2.5 quarts per minute, but can be reduced for smaller fish by nippling the bottom of the inlet pipe with a soft rubber hose.

Discussion

The polyethylene-bottle unit has proved adequate to speed up the selective breeding program for trout at the Benner Spring Fish Research Station. These bottles have been used also for exploiting the

FIGURE 3.--Swim-up fry in bottle. Daily treatment of formaldehyde eliminated the need to remove dead eggs or disturb fry. Note the dead eggs on the bottom.

differences in spawning techniques, testing the effects of nutrition on brood stock, and determining the effect of radiation on trout eggs, as well as in other experiments in which many individual lots were needed.

In all cases, this unit has proved effective and economical. The cost of polyethylene bottles is much less than that of specially manufactured containers. As the required amount of water is reduced, it is easy to filter the supply, if required, before it flows over the eggs.

Although this unit has been used principally for trout eggs and fry, eggs of some warm-water fish species have been incubated and hatched by this method. Eggs of the chain pickerel (Esox niger) were incubated and allowed to swim up in this unit. Eggs of the northern pike (E. lucius), muskellunge (E. masquinongy), and walleye (Stizostedion vitreum) were also eyed in it. However, the effect of formaldehyde on the fry has not yet been determined.

The large jars (Buss 1959b) have been useful in the diet experiments, particularly for starting diets. This unit makes it possible to replicate the experiments in a very limited space. Experiments have shown that differences in diets are detected as well in these jars as in tanks or troughs. Because each jar can have individual chemical treatment, it is possible to test fungicides, disease treatments, and the various concentrations of these chemicals, all in one unit.

Although the fish in these jars become very concentrated (figure 4), they do not exhibit any fin wear or other external signs usually associated with crowded fish. This phenomenon cannot easily be explained, though it may be noted that the glass jars have very smooth sides. Normally fin wear is associated with crowded fish, rather than with the physical makeup of the containing unit.

In conclusion, it can be stated that the units described here have been effective in trout research and in providing identical environments when necessary. There are also indications that these methods can be adapted for research on warm-water fish. The limited space and water requirements make it possible to accomplish a maximum amount of research in a limited space in hatcheries or laboratories.

FIGURE 4.--A photograph taken 90 days after 300 brook trout swim-up fry weighing 1 ounce were placed in this jar. The 296 fingerlings remaining weighed 1.5 pounds.

Acknowledgment

The writers wish to thank Mr. Daniel Reinhold for the photographic work, and other members of the staff at the Benner Spring Fish Research Station for ideas and suggestions.

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A BACK-PACK SHOCKER FOR COLLECTING LAMPREY AMMOCOETES

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and
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IN 1958, THE BUREAU OF COMMERCIAL FISHERIES began to use specific larvicides for sea lamprey control in streams tributary to the Great Lakes. The successful application of larvicides required treatment of all areas of the streams infested by sea lamprey ammocoetes. Intensive surveys were needed to determine distribution of the larvae. The use of electrical methods to determine larval distribution has been described (Stauffer and Hansen 1958). A light portable shocker was needed for these surveys because of the difficulty of access to some of the watersheds tributary to Lake Superior.

Portable shockers with converters were used for less accessible streams. The converters supplied 110-volt alternating current from 6- or 12-volt auto batteries (Tibbles 1959). The output of these converters was applied to the larval beds by two 6- by 12-inch sheet-metal electrodes. Fine-mesh scap nets were used to collect the ammocoetes shocked. These shockers, as used in the past, were heavy (60 to 70 pounds) and difficult for one man to carry over rough terrain.

A lighter, more portable unit was designed for use with a 6-volt converter and a light-weight battery. Motorcycle batteries had been used previously for shocking fish (Haskell, MacDougal, and Freeman, 1954). Several types were investigated and found suitable. A 6-volt battery, 6-1/2 by 6-1/2 by 3-7/8 inches, weighing 13 pounds and having a capacity of 30 ampere-hours, was chosen. The use of this battery with a 6-volt converter reduced the weight of the shocker to about 25 pounds.

Two methods for carrying the shocker were developed (figures 1 and 2). The frame on which the unit is attached (figure 1) is made of 1/4-inch marine plywood, mounted on a light packboard. In figure 2, the converter and the battery box are mounted on 1/4-inch plywood backed with 1/2-inch foam rubber. The battery box is constructed of sheet aluminum. No packboard is needed with this unit. The only difference between the two methods is the slightly better weight distribution of the second.

A disadvantage of wet-cell batteries in any portable shocker is leakage of battery acid. Liquid rubber painted on the inside of the battery box protects the plywood or sheet aluminum from acid corrosion and also protects the converter mounted under the battery (figure 1). A plastic bag enclosing the battery furnishes additional protection.

The electrodes and scap nets are combined for further improvement of portability. A 4-foot length of 1-inch dowelling serves as a handle. Each electrode is a frame of 1/8-inch welding rod, 8 by 8 inches, covered with fine-mesh copper screen held in place by solder (figure 3).

This study is part of a program conducted by the Bureau of Commercial Fisheries under contract with the Great Lakes Fishery Commission.

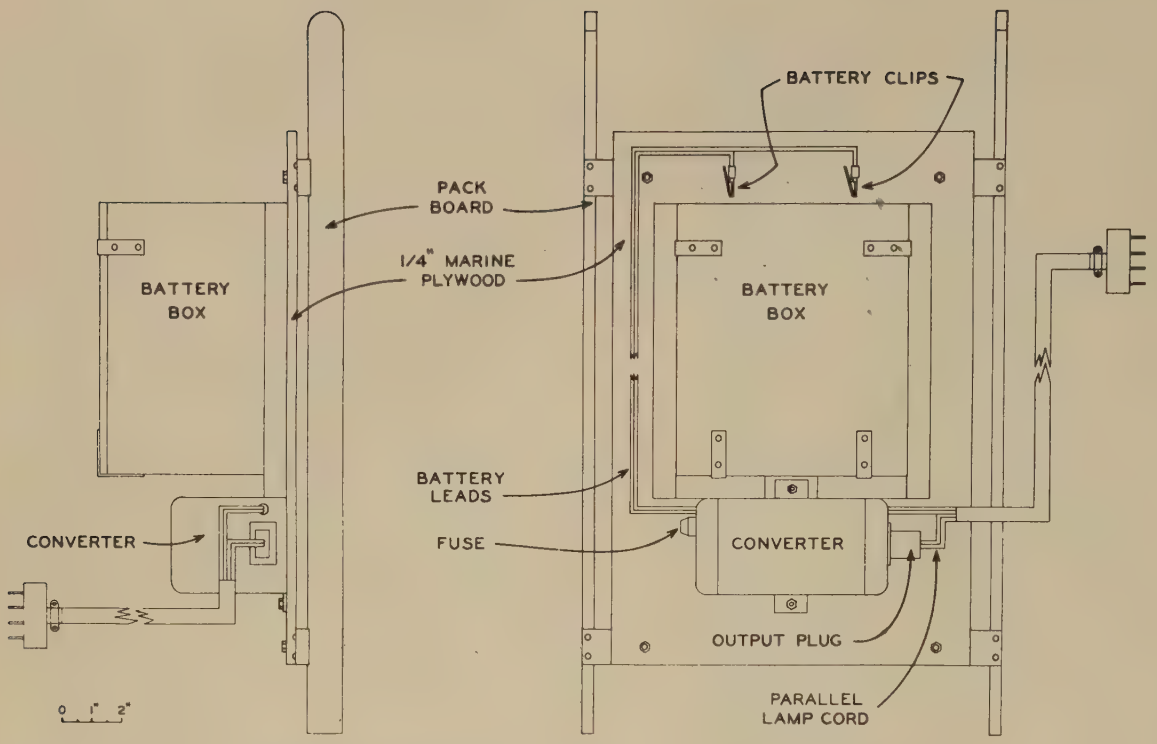


FIGURE 1.--The method used to carry the back-pack shocker.

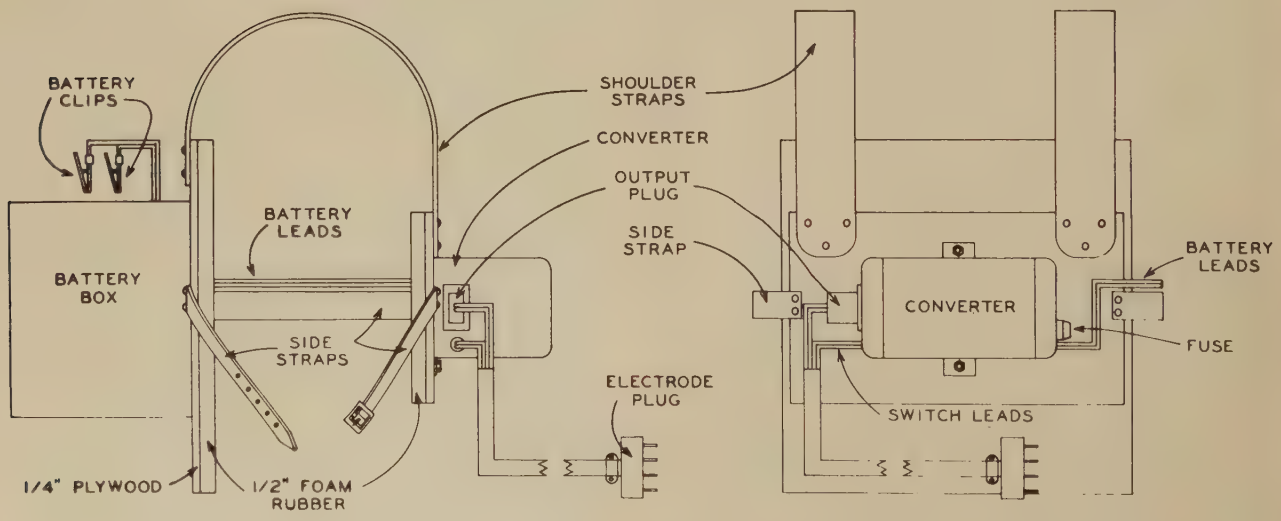


FIGURE 2.--A modification of the method of carrying the back-pack shocker.

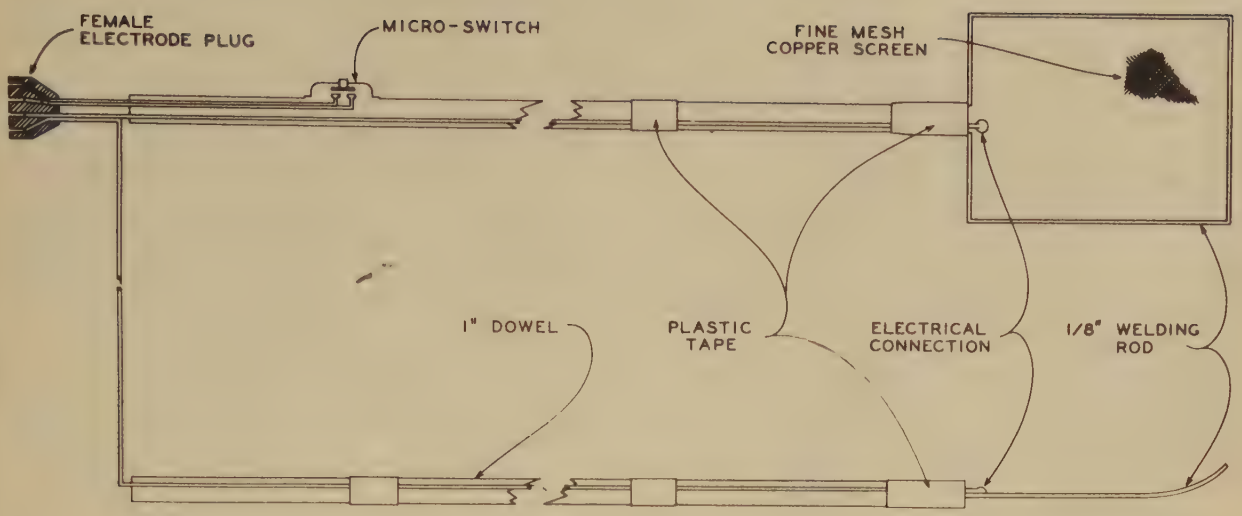


FIGURE 3.--Electrode scap nets used with the back-pack shocker.

The costs of the two units, including labor, are similar--less than $70 at wholesale prices.

The collection of lamprey ammocoetes is more effective with this shocker if the output is interrupted. A continuous current paralyzes the ammocoetes in their burrows, except at the fringe of the electrical field. Therefore, the on-off switch was removed from the converter and replaced with a snap-action switch mounted on one of the electrodes. Power to the converter is interrupted by manual operation of this switch. Interruption of the battery rather than the converter output prolongs the life of the battery. The 6-volt, 30-ampere-hour battery does not have the life of larger batteries and needs frequent charging. Four hours is about maximum use for each charge.

The short service hours of the 6-volt battery made it necessary to have a battery charger in the field. A charger was devised from the generator in the 12-volt ignition system of the survey vehicle. (Figures 4 and 5 are schematic diagrams of this circuit.) The battery lead from the voltage regulator to the starting solenoid and the starter-switch lead to the same terminal must be removed and connected to the center lead (terminal No. 2) of the single-pole, double-throw switch installed on the charger panel (figure 4). Terminal No. 1 of the switch is then connected to the solenoid terminal where the two wires have been removed.

A change must be made in this circuit if the vehicle has a positive ground. The ammeter leads and the polarity of the shocker-battery charging leads must be reversed. If the vehicle has a 6-volt battery system, the 3-ohm, 150-watt rheostat (figure 4) must be changed to a 2-ohm rheostat of 50- to 100-watt capacity.

The charging circuit with the rheostat in the zero position will deliver in excess of 5 amperes to the shocker battery if the battery charge is very low. In this situation, the rheostat should be used to lower the charging rate to 5 amperes. During long periods of charging, however, the rate of charge should not exceed the specified charge rate of the shocker battery.

The battery is connected to the charging circuit during travel between stations. When travel time between stations is not sufficient to recharge the battery, and if 110-volt current is available, a standard 6-volt, 2-ampere battery charger is used to supplement the vehicle charger.

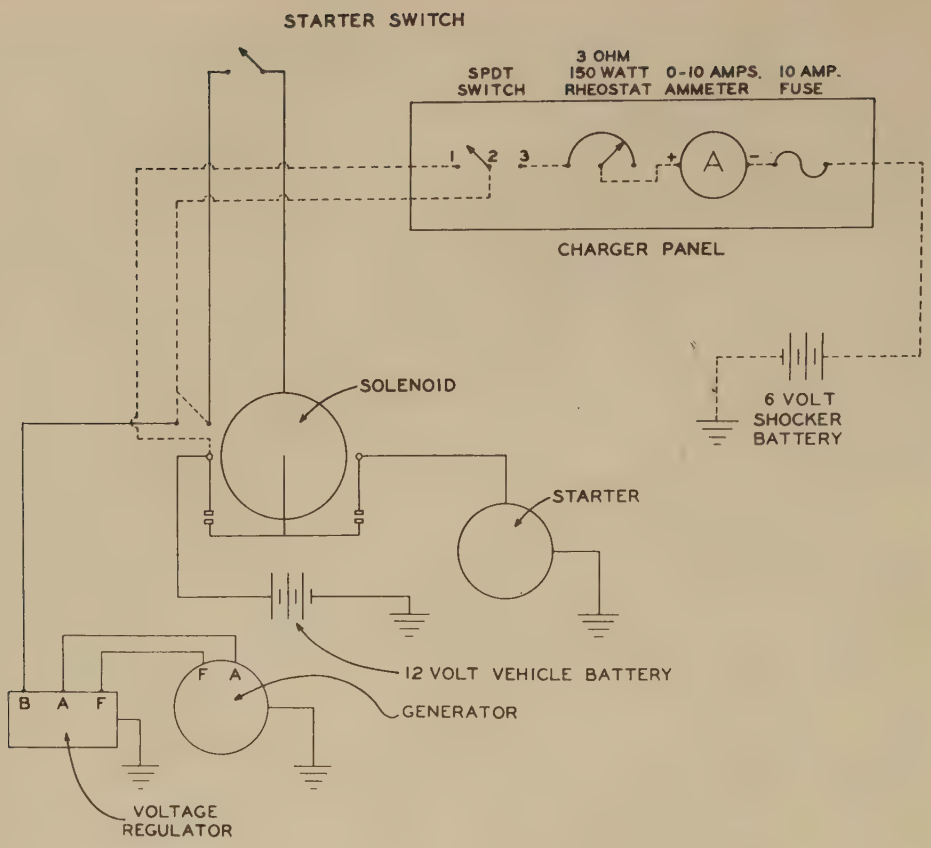
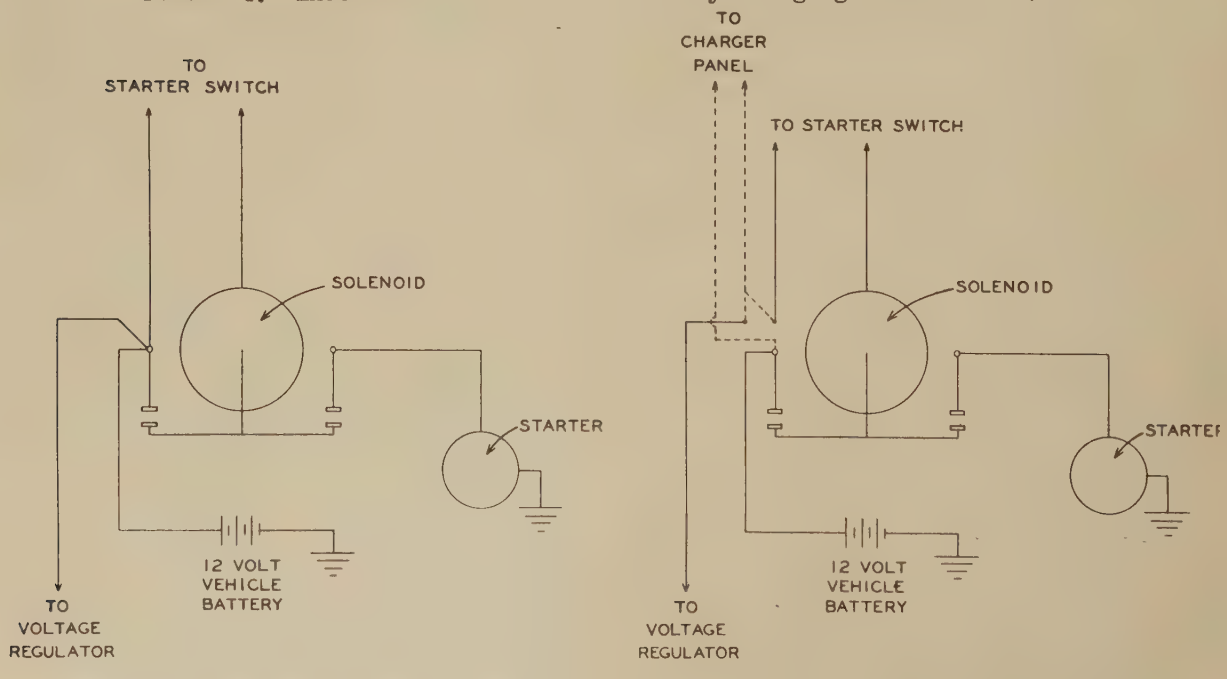


FIGURE 4.--Installation of shocker battery-charging circuit and panel.



SOLENOID BEFORE INSTALLING CHARGE CIRCUIT

SOLENOID AFTER INSTALLING CHARGE CIRCUIT

FIGURE 5.--Typical vehicle solenoid before and after installation of charge circuit.

The back-pack shocker has the usual limitations of electrical shockers with respect to water conductivity, bottom conductivity, and the ratio of the two. Its portability, however, permitted the shocking of more larval habitat and thus made possible the collection of more ammocoetes in less time and with less effort than other types of shocker require. This shocker has proved effective also for collecting fish on smaller streams, but is of limited value in large streams because the electrical field is relatively small.

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EMERGENCE AND MORTALITY OF CHINOOK SALMON FRY IN A NATURAL REDD

Late in August 1957, a chinook salmon redd was located and marked in the Lemhi River near Leadore, Idaho. Spawning was at its peak around September 1. Several adult females spawned on the same riffle, and some redds may have overlapped.

On December 12, a small section of the gravel was dug from the spawning riffle and 34 sac fry (9 of them dead) were collected. Water temperature at midday was 38° F. Hatching time was believed to have been around the first of December.

On January 21, 1958, a specially made trap was put over the redd to collect fry emerging from the gravel. Fry dug from the gravel then showed considerable development of fins, pigmentation, and absorption of yolk sac, though their length (35 millimeters) had not increased. The first emergents were collected on February 15, about 10 weeks after hatching. Emergence continued till March 4. (See table.)

RATE OF EMERGENCE OF CHINOOK SALMON FRY FROM A NATURAL REDD IN THE LEMHI RIVER, 1958

Date of checking trap	Number of fry	Water temperature °F.
January 21---	(Trap installed)	37
January 30---	0	37
February 8---	0	36
February 15--	4	38
February 19--	88	37
February 23--	37	43
February 28--	70	37
March 4------	13	40
March 9------	0	38
March 17-----	1	40
Total-----	213	--

On March 17, when the gravel under the trap (4 feet square) was dug, 104 live fry (yolk sac absorbed), 46 dead fry (yolk sac absorbed), 5 dead sac fry, and 136 dead eggs were collected. The live fry found in the gravel seemed thinner and weaker than those that had emerged. Assuming that these fry would not have emerged because of their weak condition, it was calculated that 73 percent of 504 eggs hatched, and 42.3 percent produced fry that emerged.

--STACY V. GEBHARDS, Idaho Fish and Game Department, Jerome, Idaho.

A FLY-ROD ELECTRODE SYSTEM FOR ELECTROFISHING

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THE SURVEY OF MANY OF THE REMOTE STREAMS in the Great Smoky Mountains National Park was undertaken during the winter of 1958-59 as part of the program of the Appalachian Sport Fishery Investigations. The objectives were to plot the distribution of the native Eastern brook trout, to check on streams which fishermen had reported to be barren, and to determine the upstream limits of populations of the exotic rainbow trout. The collections of fish were made with electrofishing gear because previous work in the Smokies had shown that all-season electrofishing was possible with a 230-volt alternate-polarity electrode system.

It was intended to have at least three men conduct the survey, but only two were available. They found it difficult, therefore, to manipulate the two-brail type of electrode system, net the fish, and handle pickup pails during the electrofishing. As most of the streams were small, the crew experimented with electrodes which could be operated by one man. The result was a fly-rod system which was developed by Zenith D. Whaley, a member of the staff.

The fly-rod electrode system, with a 230-volt back-pack power unit mounted on a packboard.

Description

The fly-rod electrode system consists of the butt section of a 2-piece, 8.5-foot fiberglass fly rod on which two lengths of No. 18, 68-strand, neoprene-covered test wire are taped (see photograph). The wires extend about 4 feet beyond the handle of the rod, and a two-conductor male plug is attached to them for connection to the power unit. The wires extend 9.5 feet from the tip of the rod and are bound together for the first 16 inches with plastic electrical tape. Beyond that, alternate 12-inch sections of the wires are peeled, with 2 inches of covered and taped wire between the peeled sections. For example, a 12-inch section of wire A is peeled; next the wires are bound with tape for 2 inches; and then a 12-inch section of wire B is peeled. In all, there are four peeled sections on one wire and three on the other. The peeled sections serve as the electrodes, and the electrical circuit is completed through the water from a bare section of one wire to the nearest

bare section of the other wire. At the end of the seventh electrode, the wires remain covered for 2 inches before they terminate. The tips are taped together in such a way that they are completely insulated from each other.

The total length of the electrode system, including the section of fly rod, is 13.5 feet. Presumably, longer systems could be used handily and effectively if needed. The materials have proved to be durable, and the total investment in them is small.

Power Supply

The fly-rod electrode system can be powered by 115-volt and 230-volt units, depending upon the resistivity of the water. We employ a 230-volt unit in all waters in which the resistivities exceed 10,000 ohms. The type of power unit used depends upon the accessibility of the streams. Because many of the streams in the Park can be reached only by foot trails, a 230-volt back-pack unit of a type described by Haskell, MacDougal, and Freeman (1954) is one of the more convenient sources of power. This unit is mounted on a packboard and carried by the operator of the electrodes. A horn-type push-button switch on the handle of the fly rod activates a relay in the power unit and enables the operator to control the flow of electric current to the electrodes.

A 115-230-volt gasoline-type generator is used with the electrode system where a stream can be approached by vehicle. A reel of two-conductor wire installed between the generator and the electrodes permits electrofishing within a 50-yard radius. If the streams were not so steep and rough, a small punt could be used to haul the generator and fish-holding containers.

Operation of the Fly-Rod System

The operator of the fly-rod electrode system wades the stream and roll-casts the electrodes into the water. The very flexible and strong test wire can be cast easily and accurately. The electrodes can be flicked around rocks, under banks and overhangs, and into or around piles of brush. They can be manipulated quickly to intercept moving and frightened fish. In the hands of a person who is a fisherman and who knows where to find fish in a stream, the fly-rod system is a highly efficient collecting tool. The coworker carries a scap net and a pickup pail, and he positions himself where he can collect the fish easily.

The fly-rod electrode system was used routinely in 1959 on small trout streams in the Shenandoah and Great Smoky Mountains National Parks. In trials, it proved to be as effective as larger alternate-polarity systems on all sizes and species of fish present. The best results, including a minimum mortality of fish, were obtained when the electrodes were powered with 230 volts and the resistivities of waters ranged from 30,000 to 40,000 ohms. The high resistivities of 75,000 ohms and greater which were encountered during winter were reduced by placing blocks of stock salt in the streams (Lennon and Parker 1958). Using the fly-rod electrode system, the two-man crew was able to sample fish populations in dozens of streams which never had been surveyed.

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AN IMPROVED INSTRUMENT TO SECTION BONES FOR AGE AND GROWTH DETERMINATIONS OF FISH

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THE AGE AND GROWTH OF SCALELESS FISHES can be determined by examining bony structures or otoliths. Often these structures first must be sectioned. An instrument that has been used to section the spines of channel catfish consists of a movable saw which is pushed into a spine held by hand in a fixed location while the thickness of the sections is regulated by eye (Leonard and Sneed 1951). A method for sectioning carp spines has been described by English (1952).

The improved instrument, herein described, consists of a fixed saw (which, however, can be elevated), a mechanical infeed table that regulates the thickness of the sections, and a sliding table to which are attached a V-block and clamp that hold immovable the structure to be sectioned. The basic design of this instrument was conceived by me; it was perfected and constructed by Mr. John Snell of the Physics Instrument Shop, University of Missouri.

Description

Figures 1 through 4 should provide ample detail for a machinist to duplicate this instrument.

An aluminum base plate, 7 by 10 by 1/2 inch, has an aluminum motor pillar and motor clamp mounted on the left side. The pillar is grooved to allow the one-piece motor clamp to be moved vertically. This allows use of saws of different diameters and is useful when otoliths are to be sectioned. The diameter of the motor clamp will vary with the diameter of the motor used. The pillar and clamp must be aligned at a 90° angle to the infeed table.

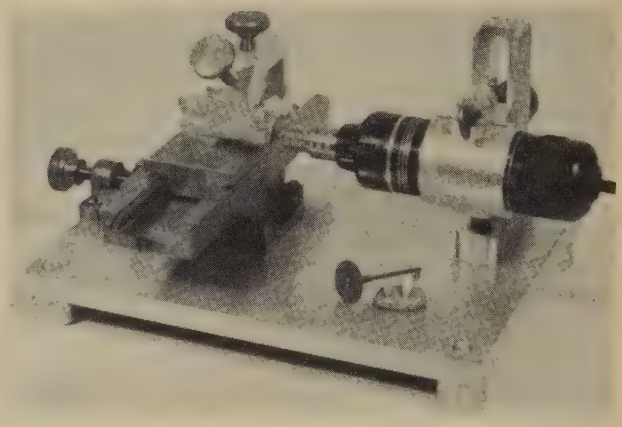

FIGURE 1.--An improved instrument to section bones.

The infeed table, made from 2- by 3/4-inch brass, consists of two parts and is located to the right and in the center of the base plate. The two parts are held together and parallel by a sliding dovetail joint. The female part of the table is fastened to the base plate. The male part can be moved by turning the brass 1/4 - 32-thread screw which was machined with a 1/8- by 1/2-inch-diameter flange at one end. The infeed screw is threaded through

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a bracket at the right of the infeed table; the flange is fitted into a suitable-sized slot in the under side of the male part of the infeed table.

One clockwise revolution of the 32-thread infeed screw moves the table 1/32 inch toward the saw; half a revolution moves it 1/64 inch toward the saw. This regulates the thickness of the section to be cut from a spine or bone. Counter-clockwise revolutions of this screw will withdraw the infeed table from the saw.

The sliding table is mounted on the male infeed table at a 90° angle. The sliding table is constructed in the same way as the infeed table but is hand-operated. A screw and washer set at each end of the female sliding table limit the travel of the male table and clamp (figure 1).

An aluminum V-block and clamp are mounted on the male sliding table at a 90° angle. An object can be clamped in the V-block in two different ways. A vertical screw forces a V-shaped wedge into the V-block, and another screw at an angle of 45° forces a rectangular plate against one side of the V-block.

The dovetails in the infeed and sliding tables can be carefully machined so that they will fit together with very little play between them, or the male portion can be machined undersized so that a 1/8-inch adjustable shim can be fitted to the female portion to take up excess play (figure 1).

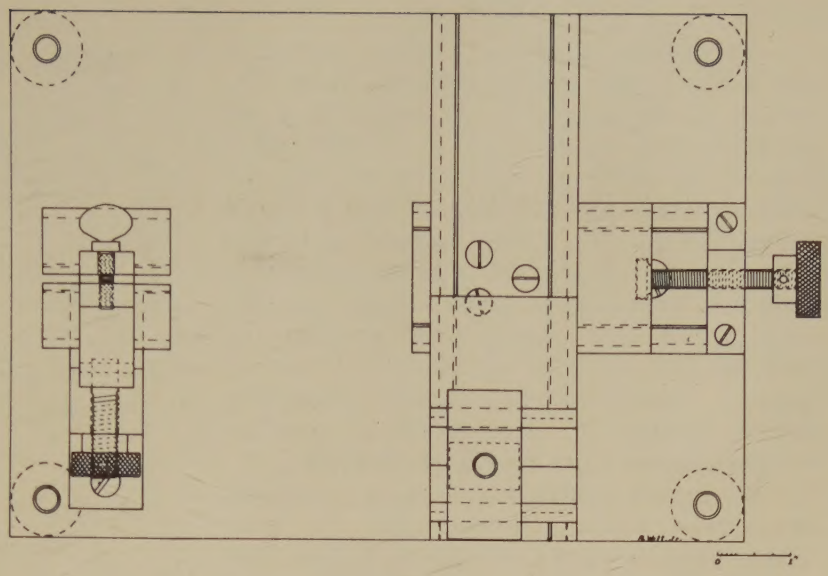

FIGURE 2.--Plan view.

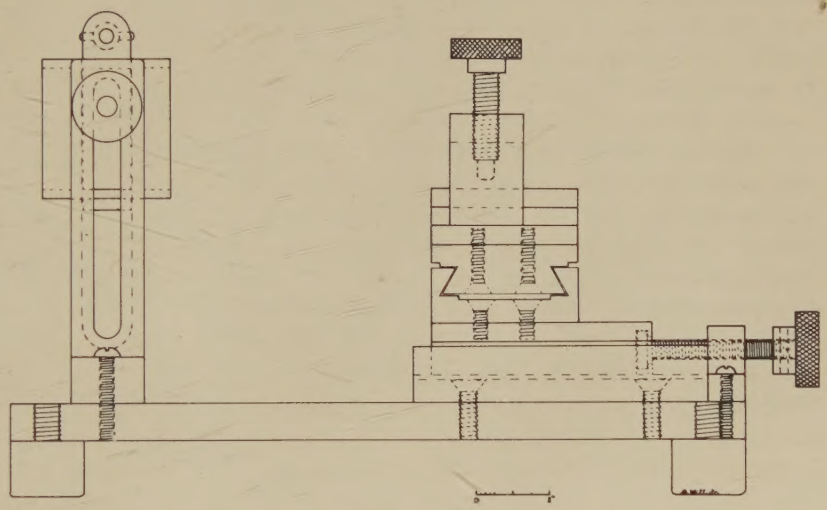

FIGURE 3.--Side view.

The saws are 7/8 inch in diameter and 5/1000 inch thick. The carborundum cutting discs are 7/8 inch in diameter and 1/64 inch thick. Both have a 5/64-inch hole and are mounted on a mandrel with a 1/8-inch-diameter shaft.

The motor and accessories may be purchased for about $25, and it will cost about $75 to construct the remainder of the instrument.

Operation

To section a spine, it is clamped in the V-block and moved toward the saw by means of the infeed screw until the point of sectioning is reached. The proximal end of the spine is cut off by moving the sliding table into the saw. The table is moved back, the infeed screw is turned one-half of a revolution, and the section is then cut. This results in a spine section that is 1/64 inch thick. The section can then be viewed under a binocular microscope. Small spines are best glued between strips of balsa wood to prevent shattering and loss during sectioning.

To section fresh-water drum otoliths, the carborundum cutting disc is used. The otolith is first fastened to a "popsicle stick" with sealing wax and then clamped on top of the V-shaped wedge in the V-block. The infeed screw is used to bring the center of the otolith in line with the cutting disc. The motor clamp is adjusted to bring the cutting disc to the surface of the stick. The sliding table is moved slowly into the cutting disc until the otolith has been cut in half. The cut and polished face of half of the otolith, set in modeling clay for firm holding, can be viewed with a binocular microscope. A slight amount of glycerin on the cut face of the otolith makes the annuli clearly visible when light is transmitted through the otolith at 90° to the optical axis of the microscope.

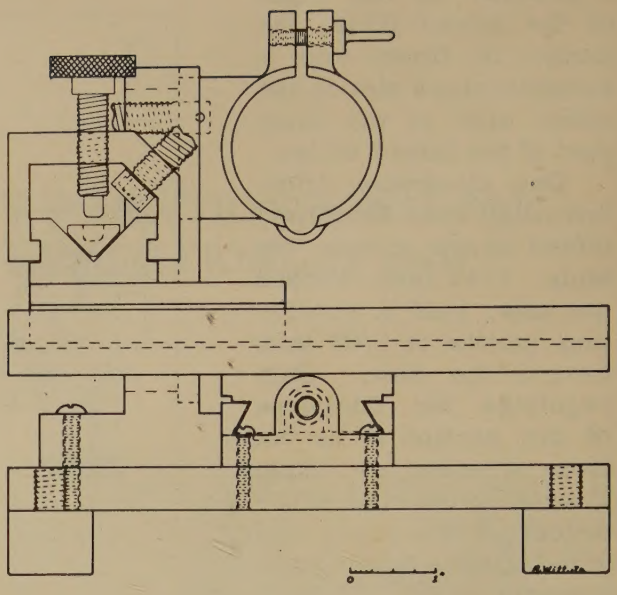

FIGURE 4.--End view.

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AN IMPROVISED DISPENSER OF THYODENE IN FIELD TITRATIONS

During early trials of thyodene as an indicator in dissolved oxygen titrations made in the field, addition of small doses (0.5 gram) of the powdered substance was difficult and apparently required more agitation than the traditional starch preparation to produce maximum coloration.

At the Mill Creek Fishery Station in California, thyodene is added to the titrate by means of a 2-ounce dropper-top polyethylene squeeze-bottle from which part of the mouse-nose end has been cut. The size of the cut necessary to dispense the desired amount of thyodene is determined by trial and error. The atomized puff of thyodene produces immediate and maximum coloration with very little agitation.

--HAROLD A. GANGMARK, Bureau of Commercial Fisheries, Seattle, Washington.

TO CONTRIBUTORS

Contributions for publication in THE PROGRESSIVE FISH-CULTURIST are always welcome. All contributions should be directed to: The Editor, THE PROGRESSIVE FISH-CULTURIST, Bureau of Sport Fisheries and Wildlife, Washington 25, D. C.

Manuscripts should be typewritten, double-spaced, on one side of white bond paper. Illustrations should be in the form of sharp, glossy, black-and-white photographs or drawings prepared with waterproof black India ink on heavy white paper or tracing cloth.

A short abstract should be submitted with each manuscript. When an article has appeared in an issue of THE PROGRESSIVE FISH-CULTURIST, the publication data will be added to the abstract and this will then be forwarded to Biological Abstracts and Sport Fishery Abstracts.

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